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# Basic principles of TLC

## Introduction to thin layer chromatography

Thin layer chromatography (TLC) and high performance thin layer chromatography (HPTLC) – now also called planar chromatography – are, like all chromatographic techniques, based on a multistage distribution process. This process involves: a suitable adsorbent (the stationary phase), solvents or solvent mixtures (the mobile phase or eluent), and the sample molecules. For thin layer chromatography the adsorbent is coated as a thin layer onto a suitable support (e.g. glass plate, polyester or aluminium sheet). On this layer the substance mixture is separated by elution with a suitable solvent. The principle of TLC is known for more than 100 years now<sup>1)</sup>. The real break-through of TLC as an analytical method, however, came about 35 years ago as a consequence of the pioneering work of Egon Stahl<sup>2)</sup>.

After some time of stagnation thin layer chromatography has gained increasing importance as an analytical separation technique, which is probably due the effects of instrumentalisation and automatisisation<sup>3)</sup>. At the same time the applicability of thin layer chromatography was enhanced by the development of new adsorbents and supports.

Today MACHEREY-NAGEL offers a versatile range of ready-to-use layers, which are the result of 30 years of continuous research and development.

### References

- 1) M. W. Beyerinck, Z. Phys. Chem. **3** (1889) 110
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## Principle steps of a thin layer chromatographic separation

### Sample preparation

For a chromatographic separation the sample must meet several requirements to obtain good results. It is not possible to go into detail here. Since the TLC plate is a disposable product, sample preparation in general is not as demanding as for the other chromatographic methods. However, eventually several steps for sample pretreatment may be necessary. These include sampling, mechanical crushing of a sample, extraction steps, filtration and sometimes enrichment of interesting components or clean-up, i.e. removal of undesired impurities.

Our TLC micro-sets introduce some simple methods of sample pretreatment. The examples of the beginner's set do not require complicated procedures. The dyes or dye mixtures chosen for this set can be applied without further preparation. The advanced sets require the user to carry out some additional steps for preparing a sample. These individual achievements in sample pretreatment introduce the user to techniques often performed in industrial laboratories.

Thorough preparation of samples is an important prerequisite for the success of a thin layer chromatographic separation. For our range of products for more demanding sample pretreatment please see the chapter "Sample preparation" from page 179.

### What can TLC/HPTLC offer today?

The success of thin layer chromatography as a highly efficient microanalytical separation method is based on a large number of advantageous properties:

- high sample throughput in a short time
- suitable for screening tests
- pilot procedure for HPLC
- after separation the analytical information can be stored for a longer period of time (the TLC ready-to-use layer acts as storage medium for data)
- separated substances can be subjected to subsequent analytical procedures (e.g. IR, MS) at a later date
- rapid and cost-efficient optimisation of the separation due to easy change of mobile and stationary phase

For a better understanding of a thin layer chromatographic separation we describe here the basic steps:

- sample preparation
- sample application
- development of a chromatogram, separation techniques
- evaluation in TLC – visualisation of separated substances, qualitative and quantitative determinations

### Sample application

The aim of a chromatographic separation determines how the sample should be applied to the TLC plate or sheet. The most frequent technique still is application with a glass capillary as spot or short streak. Application as streak will yield better results especially for instrumental quantification. For both types of application some manual skill is required to obtain reproducible results. Substance zones which are too large from the beginning will cause poor separations since during chromatography they will become even larger and more diffuse.

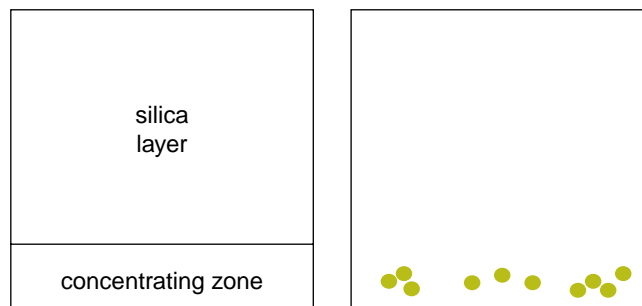
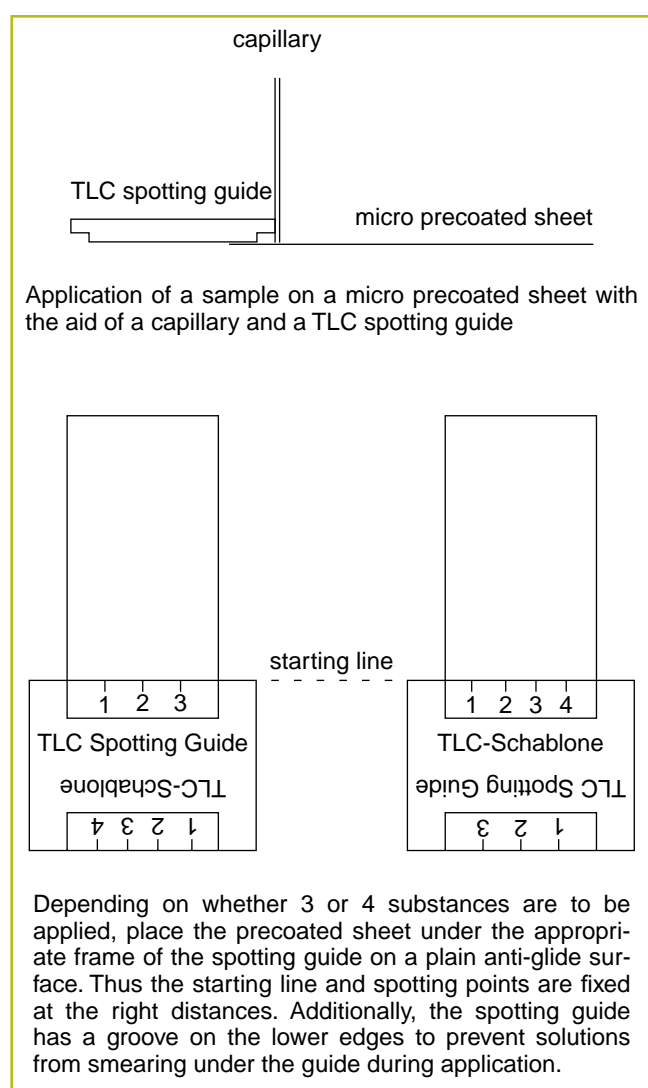
Our TLC micro-sets for example use this very simple way of application. The mixture to be separated and the reference solution are applied to the micro pre-coated sheets as spots by means of glass capillaries. Only use each capillary once to avoid contamination of the following samples. The capillaries fill themselves quickly when dipped into organic sample solutions, with aqueous solutions filling will be much slower; in some cases it may be necessary to use a rubber cap. Before emptying the capillary roll the submerged end horizontally on filter paper. If the capillary is filled to the upper end, however, you may apply the "clean" upper end to the adsorbent layer. Place the capillary on the layer vertically and carefully, vertically so that the capillary empties itself and



## Principle steps of a thin layer chromatographic separation

carefully to avoid damage to the layer. Damaged layers result in unevenly formed spots. To keep spots as small and compact as possible, it is advisable to apply a solution in several portions with intermediate drying (blow with cold or hot air). This is especially important for aqueous sample solutions.

To facilitate application we recommend a spotting guide designed for our micro precoated sheets. For other plate sizes suitable spotting guides are commercially available. The following figures demonstrate the clean and easy application of samples with the above-mentioned spotting guide.



DC plate SILGUR with concentrating zone



A valuable aid for manual application especially of large volumes of very dilute samples is the concentrating zone (e.g. SILGUR-25 UV<sub>254</sub>), which consists of a chromatographically inactive adsorbent (kieselguhr). The substances to be separated are concentrated to a small band at the interface between concentrating zone and the chromatographically active adsorbent (silica).

Another method for sample concentration is a short pre-elution (few mm) with a solvent, in which all substances have a high  $R_f$  value.

If a quantitative evaluation with a TLC scanner is to follow the separation we recommend that you use commercially available sample applicators for spotting. These range from simple spotting guides via nanoapplicators to completely automated spotting devices. Application as streak can be performed automatically by spraying of the sample without touching the layer of the TLC plate. Application as band over the whole width of the TLC plate is especially important for preparative TLC.

After application allow the solvent of the samples to evaporate completely (about 10 minutes) or blow with cold or hot air. Development of a chromatogram should never start before the solvent of the applied samples is evaporated completely.

# Basic principles of TLC

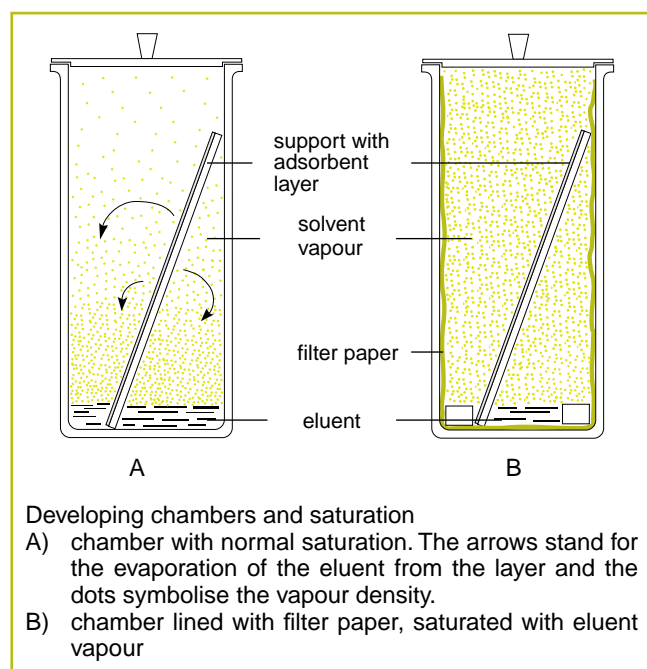
## Principle steps of a thin layer chromatographic separation

### Developing a chromatogram – separation techniques

The most frequently used separation technique is ascending TLC in the customary trough chamber (standard method, linear development). Usually it is applied as single development. However, multiple development, with or without change of eluent (step technique) can improve separation results. For 2-dimensional development only 1 spot of the sample is applied in one edge of a plate. After chromatography in the first direction the plate is dried, turned by 90° and developed in the 2nd dimension with another eluent. Thus complicated mixtures give 2-dimensional chromatograms taking advantage of the different separating properties of two eluents.

For selection and optimisation of the eluent numerous publications are available. A generally applicable standardised optimisation method is described by H. Keuker et al.<sup>3)</sup>

It is important to pay attention to the atmosphere in the developing chamber. If reproducible migration distances are required, saturation of the chamber atmosphere with eluent vapour is necessary. For this purpose the developing chamber is lined with well absorbing chromatography paper (e.g. MN 260) and charged with a correspondingly larger volume of eluent.



A better control of chromatographic conditions, especially of the vapour phase, is achieved in flat chambers (horizontal chambers) or a trough with sandwich configuration. In these techniques the vapour phase is excluded by a covering plate. As in classical TLC eluent supply is linear.

With continuing instrumental development (based on the wick circular development of paper chromatography) one can also use circular development, i.e. the eluent is led from the middle of the plate to the outside. Due to the relation<sup>4)</sup>

$$R_f^{\text{linear}} = R_f^2{}^{\text{circular}}$$

the lower  $R_f$  range of linear development, which has its optimal  $R_f$  values between 0.2 and 0.7, is considerably expanded resulting in increased resolution. Operated with commercially available instruments this technique allows a decrease in developing time, the possibility to work under inert atmosphere and a simple optimisation of eluent flow rates. The opposite development, i.e. anti-circular, where the eluent is fed from the outside to the middle, is extremely fast and allows a better resolution of the upper  $R_f$  range.

Acceleration of the separation and utilisation of longer separation distances can be obtained with "forced flow" techniques. Thus with OPTLC (over pressure thin layer chromatography) the plate (or sheet) is sealed with a frame and the eluent is forced through the covered layer by pressure. Rotation techniques utilise centrifugal forces. This CLC (centrifugal layer chromatography) comprises the whole range from analytical to preparative separations and even substance collection via continuous flow techniques. Another technique is circular development under high pressure, called high pressure planar liquid chromatography (HPPLC)<sup>5)</sup> with extremely short developing times and optimal flow rates. As an example: on CHIRALPLATE D,L-amino acids are separated into their antipodes within 2 – 3 minutes – an unsurpassable proof for the capabilities of TLC. For all forementioned techniques today commercial instruments are available.

Another very interesting technique is the PMD technique<sup>6)</sup> (Programmed Multiple Development), which is a true gradient development on silica for thin layer chromatography. Contrary to the common multiple development every single run is slightly longer than the previous run. Thus broadening of the substance zones during the chromatographic process is easily compensated for. Usually, about 10 to 25 development cycles are run, generally with a universal gradient. Since this technique has been automated in the meantime, you can also find the name AMD (Automated Multiple Development)<sup>7)</sup> (also see our nano plates with extremely thin silica layer, page 271).

However, it should be noted, that the considerable increase in performance with these techniques also requires a considerable increase in instrumental expense.



## Principle steps of a thin layer chromatographic separation

### Evaluation of a thin layer chromatogram

The evaluation depends on the purpose of a chromatographic analysis. For qualitative determination often localisation of substances is sufficient. This can be easily achieved by parallel runs with reference substances.

A parameter often used for qualitative evaluation is the  $R_f$  value (retention factor) or the 100fold value  $hR_f$ . The  $R_f$  value is defined as follows:

$$R_f = \frac{\text{distance starting line} - \text{middle of spot}}{\text{distance starting line} - \text{solvent front}} = \frac{b}{a}$$

i.e. the  $R_f$  values are between 0 and 1, best between 0.1 and 0.8 (i.e. 10 – 80 for  $hR_f$ ). If reproducible  $R_f$  values are to be obtained it is, however, essential that several parameters such as chamber saturation, constant composition of solvent mixtures, constant temperature etc. are strictly controlled.

A quantitative evaluation is possible by suitable calibration measurements. For this purpose either the area of a substance spot is measured or a photometric evaluation is performed directly on the layer. The latter procedure, however, requires a higher instrumental expense.

The following paragraphs describe the most frequently used methods for evaluation in TLC.

### Qualitative detection

Qualitative evaluation is generally made directly on the TLC plate via the characteristic  $R_f$  values of substances, i.e. the ratio of distance start – substance zone to distance start – solvent front and specific chemical reactions.

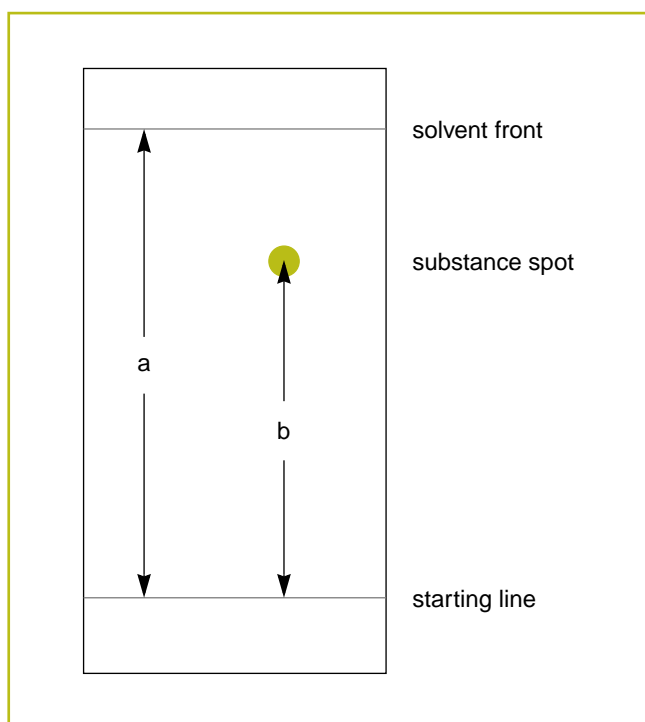
### Visualisation of separated substances

First of all it is necessary to recognise the position of a substance spot. Only in very few cases the sample is a dye which can be seen with the naked eye. Much more often for unspecific visualisation substances can be viewed under UV light, since many substances show a UV absorption. If a fluorescent indicator is added to the layer, all substances absorbing in the respective region of wave length cause a quenching of the fluorescence, i.e. they appear as dark spots on the fluorescent layer. Customary fluorescent indicators are excited at 254 nm or (less frequently) at 366 nm with a mercury lamp. For our programme of fluorescent indicators for TLC please see page 299.

The identification of separated substances is possible via the  $R_f$  value compared to the pure compound, which is often applied simultaneously on the same plate.

For a number of compounds their native fluorescence can be used for visualisation, which is excited by UV light (mostly long-wave UV) (e.g. aflatoxins). This allows not only determination of the  $R_f$  value, but often enables a further qualitative assignment.

If these methods do not allow localisation or characterisation of substances, especially post-chromatographic detection methods can be applied, chemical reactions on the TLC plate<sup>8)</sup>. Quite unspecific reactions are iodine adsorption and the charring technique (spraying with sulphuric acid and heat treatment).



# Basic principles of TLC

## Principle steps of a thin layer chromatographic separation

More reliable results are possible with specific reagents for spraying or dipping, which form coloured or fluorescent compounds with the substances to be detected. Depending on the sensitivity of these reactions they are not only used for group or substance specific characterisation (in addition to the  $R_f$  value) but also for quantification down to trace levels. As example take the ninhydrin reaction. Formation of a (usually red) zone with this detection method yields the information, that a certain group of substances, e.g.  $\alpha$ -amino acids, are present. The  $R_f$  value allows further assignment to one or several single compounds.

For identification of a substance a combination of different detection methods can be useful. Thus almost all lipids can be converted to products with light green fluorescence by reaction with 2',7'-dichlorofluorescein. Adsorption of iodine vapour enables a differentiation between saturated and unsaturated lipids or lipids containing nitrogen. And finally the  $R_f$  value is a third means of identification. The versatility of chemical reactions on the TLC plate after separation, which help find and characterise a substance, is almost unlimited and not nearly investigated to completion. For a small number of detection reagents and the description of some important detection reactions please see the chapter "Visualisation reagents" from page 300.

Here are some general remarks concerning spraying: **use all spray reagents under a fume hood.** The developed, dried TLC plate or sheet is placed on a sheet of filter paper for spraying. Usually it is sufficient to fill the sprayer with about 5 – 10 ml solution. Spray from a distance of about 15 cm with the aid of a rubber ball or – if available – with pressurised air. It is always better to spray a layer twice very thinly and evenly (with intermediate drying), than to saturate the layer with excessive spray reagent. In the latter case spots tend to become diffuse.

After visualisation mark outlines of the zones with a lead pencil, because some spots tend to fade after a while.

Especially for quantitative evaluation short dipping of the layer in the respective reagent solution is recommended. For this purpose automatic instruments are commercially available, which allow reproducible dipping.

When a substance is localised on the TLC plate (e.g. in the UV), but not yet identified, modern instruments for evaluation, so-called scanners, allow recording of UV spectra of individual substance zones directly on the layer, or the zone is removed by scratching or cutting (for sheets), eluted and further analysed, e.g. by FT-IR, RAMAN, NMR or mass spectroscopy.

If it is known prior to a separation, that recovery of substances (qualitative or quantitative) can cause problems, prechromatographic treatment is also possible, i.e. chemical reaction prior to the separation. Thus amino acids can be derivatised and e.g. separated as dansyl derivatives. On the developed chromatogram zones can be recognised directly by their fluorescence. One important advantage of TLC is that corresponding pre-chromatographic derivatisation reactions can be performed directly on the plate (starting zone). Since it is desirable to enhance selectivity as well as detection sensitivity by fluorescence reactions, reaction chromatography constantly gains importance.

## Quantitative evaluation

Often TLC is considered to be only a semiquantitative analytical procedure. This is true for visual evaluation of spots, since the eye can only compare but not measure absolute values. If, however, a direct optical evaluation ("in situ" measurement) is performed on the TLC plate with a thin layer scanner, the label semiquantitative is no longer valid. After measurement of calibration functions exact quantitative results are possible. Such commercial scanners offer many features such as evaluation in absorption and fluorescence, unattended programmed scanning of lanes, multi-wave length measurement, background correction, selectable base line for integration, recording of spectra, evaluation of circular or anti-circular chromatograms with very high ease of operation. In addition to manual operation control by a computer is possible with respective data collection and storage. Usually wavelengths from 200 to 700 nm are available (visible and UV), e.g. all post-chromatographic (and of course all pre-chromatographic) visualisation procedures are evaluated with the proper wavelength, which is determined with the instrument. Time requirements for all these possibilities are extremely low. Interlaboratory experiments with standard deviations of 2%<sup>9)</sup> show how excellent results are obtainable.

This is the (commercial) state of the art. Faster evaluations with more information and higher accuracy seem possible by one or several views of the total chromatogram with an optic (direct image processing<sup>9)</sup>). Data collection and evaluation are then performed by a computer.

Compared to these direct procedures for evaluation, the indirect methods for quantification, which require transfer of the substance spot (scratching, elution) from the plate to another technique, clearly loose importance.

The detection sensitivity of TLC, qualitatively as well as quantitatively, depends heavily on the pre-chromatographic or post-chromatographic sample treatment and with fluorescence measurement reaches at least the picogram level.

## References

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- 8) H. Jork, W. Funk, W. Fischer, H. Winner, Dünnschicht-Chromatographie, VCH Verlagsgesellschaft, 1989
- 9) Planar Chromatography, Volume 1, edited by R. E. Kaiser, Dr. Alfred Hüthig Verlag, Heidelberg, 1986



## Summary of MN ready-to-use layers for TLC

### Quality? – We are our most crucial critics!

Due to a stringent production control we can guarantee the continuous high quality of our TLC plates and sheets. Thus our ready-to-use layers are subjected to standardised lot tests. The surface of the layer is checked for roughness or cracks in incident and UV light. Additionally, the hardness and adherence of the layer is checked by developing the plates in solvents of very different polarities.

### Ready-to-use layers for TLC / HPTLC

Thin layer chromatography is used for a wide range of analyses, and over the years it has become apparent, that there is no universal plate which meets all possible demands. For this reason our range of TLC ready-to-use layers is very versatile to cover many different types of applications.

Our ready-to-use layers offer several advantages:

- They are immediately **ready for chromatographic separation**. Tedious coatings or impregnations are not necessary.
- They have **homogeneous, smooth, well adhering layers**, an important criterion especially for reproducible quantitative evaluation.

### Adsorbents used for MACHEREY-NAGEL ready-to-use layers for TLC

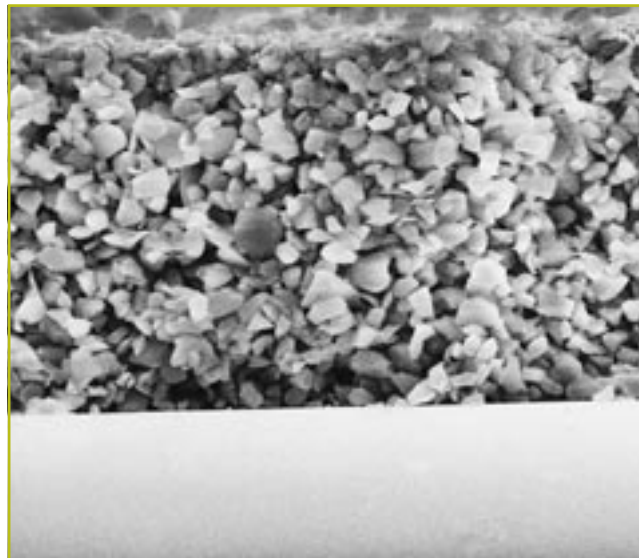
The adsorbent used most frequently in TLC is silica 60 (mean pore diameter  $60 \text{ \AA} = 6 \text{ nm}$ ). About 80% of all TLC separations are performed with this separation medium. Other customary adsorbents are aluminium oxide and cellulose. Kieselguhr, ion exchangers and polyamide complete the picture of classical adsorbents. Later on, derived from high performance liquid chromatography (HPLC) reversed phases were introduced, mainly  $C_{18}$  (octadecyl) modified silica.

A highlight of stationary phase development was production and commercialisation of a TLC glass plate for enantiomer separation in 1985 by MACHEREY-NAGEL (CHIRAL-PLATE). Now ready-to-use layers with cyano-, amino-, diol and RP-2 modified silica coatings are also available. Special layers for specific separation problems complete our versatile range of TLC plates.

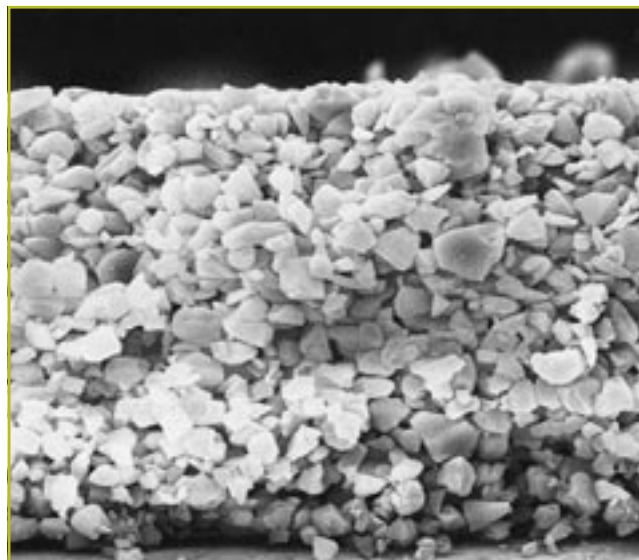
In addition to the type of adsorbent its particle size distribution and the thickness of the layer are important. For the standard silica plate the particle size is between  $5$  and  $17 \mu\text{m}$ . The thickness of the layer is  $0.25 \text{ mm}$  for analytical plates. The same adsorbent is used for preparative layers of  $0.5 \text{ mm}$  and  $1 \text{ mm}$  thickness, while for  $2 \text{ mm}$  layers a slightly coarser material is used. For high performance thin layer chromatography (HPTLC)  $0.2 \text{ mm}$  layers with a mesh size of  $2 - 10 \mu\text{m}$  are applied.

While for silica preparative layers up to  $2 \text{ mm}$  are possible, layer thickness is limited to  $0.5 \text{ mm}$  for cellulose and  $1 \text{ mm}$  for aluminium oxide.

Almost all of our ready-to-use layers are available with or without fluorescent indicator.



Electron microscope photograph of a cross section through a glass plate with silica layer magnification x 500



Electron microscope photograph of a cross section through an aluminium sheet with silica layer magnification x 500

# Ready-to-use layers for TLC

## Summary of MN ready-to-use layers for TLC

Layer	chromatographic principle	typical applications
<b>Standard layers for TLC and HPTLC</b>		
<b>unmodified silica</b>		
standard and nano silica, also with concentrating zone high purity silica 60	NP chromatography	most frequent application of all TLC layers  aflatoxins
<b>modified silica</b>		
Nano-SIL C 18	RP chromatography	aminophenols, barbiturates, preservatives, nucleobases, PAHs, steroids, tetracyclines, phthalates plant constituents, steroids
RP-18 W/UV <sub>254</sub> wetttable	NP and RP chromatography	
RP-2/UV <sub>254</sub> (silanised silica)	NP and RP chromatography	nucleotides, pesticides, phenols, purine derivatives, steroids, vitamins, sulphonic acids, carboxylic acids, xanthenes
amino-modified silica Nano-SIL NH <sub>2</sub>	anion exchange, NP and RP chromatography	
cyano-modified silica Nano-SIL CN	NP and RP chromatography	pesticides, phenols, preservatives, steroids
diol-modified silica Nano-SIL DIOL	NP and RP chromatography	steroids, pesticides, plant constituents
<b>aluminium oxide</b>		
	adsorption chromatography due to polar interactions	alkaloids, steroids, terpenes, aliphatic and aromatic compounds
<b>cellulose</b>		
unmodified cellulose	partition chromatography due to polar interactions	amino acids and other carboxylic acids as well as carbohydrates
acetylated cellulose	depending on acetyl content transition from NP to RP chromatography	anthraquinones, antioxidants, polycyclic aromatics, carboxylic acids, nitrophenols, sweeteners
cellulose ion exchangers	anion exchange	amino acids, peptides, enzymes, nucleic acid constituents (nucleotides, nucleosides) etc.
mixed layers cellulose DEAE/cellulose HR	ion exchange	mono- and oligonucleotides in nucleic acid hydrolysates
<b>polyamide</b>		
	partition chromatography due to polar interactions (e. g. hydrogen bonds)	phenolic and polyphenolic natural substances, amino acids, carboxylic acids, aromatic nitrocompounds
<b>TLC layers for special applications</b>		
<b>special silica layers</b>		
silica G, impregnated with ammonium sulphate		surfactants, lipids (neonatal respiratory syndrome)
silica, impregnated for the separation of PAHs: Nano-SIL-PAH	charge-transfer complexes	polycyclic aromatic hydrocarbons (PAHs) according to German drinking water specifications (TVO)
RP silica with chiral selector: CHIRALPLATE	enantiomer separation based on ligand and exchange chromatography	chirale amino acids, $\alpha$ -hydroxycarboxylic acids, other compounds, which form chelates with Cu(II)
<b>IONEX ion exchangers</b>		
silica / ion exchanger mixed layers	cation or anion exchange	amino acids, nucleic acid hydrolysates, aminosugars, antibiotics, inorganic phosphates, cations; racemate separations in peptide syntheses
<b>kieselguhr</b>		
commonly impregnated	RP chromatography	aflatoxins, herbicides, tetracyclines
<b>mixed layers:</b>		
aluminium oxide / acetated cellulose	NP and RP chromatography	polycyclic aromatic hydrocarbons(PAHs)
cellulose / silica	NP chromatography	preservatives
kieselguhr / silica	NP chromatography, reduced adsorption capacity compared to silica	carbohydrates, antioxidants, steroids, photographic developer substances



## Summary of MN ready-to-use layers for TLC

### Supports for TLC ready-to-use layers

The support used most often is the **glass plate**. It is resistant and easy to handle. Disadvantages are fragility, a relatively high weight (glass plates are about 1.3 mm thick) and necessary additional packing material. Also the production costs for coating glass plates are higher than for polyester or aluminium sheets. For these reasons precoated glass plates are the most expensive ready-to-use layers for TLC.

**Polyester sheets** (about 0.2 mm thick) – known under the trade name POLYGRAM® precoated sheets – can be more economically coated, since they can be manufactured in roll form. Other advantages are that polyester sheets are unbreakable, resistant towards all common solvents, need less packing and less shelf space for storage. Furthermore, they can be cut with scissors into any required size, and spots can be cut and eluted etc. Small sheets, such as 8 x 4 cm, can be economically manufactured and packed. Charring techniques can be applied for silica coated POLYGRAM® sheets, however at somewhat lower temperatures than on glass. The maximum temperature for POLYGRAM® sheets is 160 °C.

Besides polyester **aluminium sheets** are used as support for TLC ready-to-use layers. We have considerably improved our ALUGRAM® sheets. Because of a thicker aluminium support (about 0.15 mm thick) they are much more stable

and easier to cut. Even a 20 x 20 cm ALUGRAM® TLC sheet has a torsional strength which is almost as good as for a glass plate. And for scanning, too, the background noise of the ALUGRAM® is about as low as for a glass plate. As with POLYGRAM® the layers feature an outstanding adherence, which allows cutting of the sheets to any size desired without fracturing of the layer. For ALUGRAM® sheets a support of plain aluminium foil is coated using the same binder system as for our TLC glass plates. For eluents containing high concentrations of water we recommend our POLYGRAM® precoated sheets. The binder system used for POLYGRAM® sheets is absolutely stable in water.

However, like POLYGRAM® precoated sheets, TLC glass plates and ALUGRAM® sheets can be sprayed or dipped with purely aqueous visualisation reagents without damage to the layer. With ALUGRAM® precoated sheets, problems might arise when using eluents, which contain high concentrations of mineral acids or concentrated ammonia (which attack the aluminium). In such cases we recommend using our glass plates or polyester sheets. Otherwise ALUGRAM® precoated sheets offer the same advantages as POLYGRAM® sheets, however, with increased temperature resistance (e.g. for charring techniques).



# Ready-to-use layers for TLC

## Standard silica · TLC ready-to-use layers

For years now many TLC separations are standardised for routine investigations in analytical laboratories. Prerequisite are TLC ready-to-use layers which give reproducible results. The following criteria must be met:

- homogeneous coating
- homogeneous thickness of layer
- high packing density
- firmly adherent layers
- consistent chromatographic properties

Our TLC glass plates and precoated sheets meet these requirements. For special applications today a number of plates and sheets are available with special layers, which will be described in detail in the following paragraphs.

The standard silica coating is one of the most frequently used ready-to-use layers for TLC. For these plates we use silica 60 with a mean pore diameter of 60 Å, a specific surface (BET) of about 500 m<sup>2</sup>/g, a specific pore volume of 0.75 ml/g and a particle size of 5 to 17 µm.

As fluorescent indicators we use manganese activated zinc silicate for short-wave UV light (254 nm) and a special inorganic fluorescent pigment for long wave UV light (366 nm).

As binder highly polymeric products are used, which are stable in almost all organic solvents and resistant towards aggressive visualisation reagents. The binder systems used for our POLYGRAM<sup>®</sup> precoated sheets are also completely stable in purely aqueous eluents. The POLYGRAM<sup>®</sup> types SIL G and SIL G/UV<sub>254</sub> on one hand and the POLYGRAM<sup>®</sup> types SIL N-HR and SIL N-HR/UV<sub>254</sub> on the other hand have different binder systems and thus show different separation characteristics. Another special feature of the POLYGRAM<sup>®</sup> SIL N-HR is the higher gypsum content compared to SIL G.

### DURASIL glass plates for TLC

In order to meet the increasing demands with respect to the wide range of separation problems, which can be solved with classical normal phase chromatography, MACHEREY-

NAGEL has developed two more families of precoated glass plates with unmodified silica. The DURASIL family is available in two types as DURASIL-25 for TLC and Nano-DURASIL-20 for HPTLC (for HPTLC plates see the next chapter from page 270). DURASIL plates differ from the well-known plates SIL G-25 and Nano-SIL G-20 by a special binder system, which results in hard, water-resistant and wettable layers with outstanding separation properties.

Like our proven TLC glass plate SIL G-25, DURASIL-25 is coated with unmodified silica 60. They are available with or without fluorescent indicator (green fluorescence, 254 nm).

If one compares the performance of the DURASIL-25 plates with the conventional SIL G-25 plates for the separation of anthraquinone dyes (see figures), one observes a similar selectivity. A closer observation, however, reveals the differences in selectivity for the different layers. For a normal phase separation of anthraquinone dyes, DURASIL-25 and SIL G-25 behave quite similar (see fig. A), while under reversed phase conditions (fig. B) the different characteristics of the two plates become obvious. While the TLC plate SIL G-25 still shows some reversed phase properties due to the still lower polarity of the binder system, DURASIL-25 is a true normal phase plate.

DURASIL-25 plates show superior separation characteristics in the fields of environmental, clinical, medical and organic analyses. Substances separated so far include

- flavonoids
- barbiturates
- bile acids
- nitroanilines
- pesticides
- steroids

[www.mn-net.com](http://www.mn-net.com)

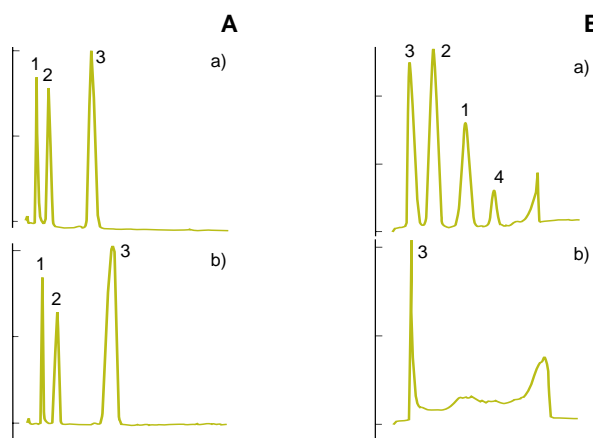
For more applications of DURASIL ready-to-use plates, check our application database on the internet.

### Comparison of DURASIL-25 and SIL G-25 for the separation of anthraquinone dyes

Layers: a) SIL G-25 UV<sub>254</sub>, b) DURASIL-25 UV<sub>254</sub>  
Eluent: A) normal phase TLC: toluene/cyclohexane (2:1, v/v)  
B) reversed phase TLC: acetone/water (1:1, v/v)  
Migration distance: A) 4.8 cm in 8 min  
B) 3.8 cm in 10 min  
Detection: TLC scanner, UV 254 nm

#### Peaks :

1. Blue 3
2. Violet 2
3. Blue 1
4. secondary spot of blue 3





## Standard silica · TLC ready-to-use layers

### ADAMANT glass plates for TLC

In addition to the well-known layers SIL G and DURASIL MACHEREY-NAGEL presents a new generation of glass plates with silica layers for TLC. ADAMANT plates feature a newly developed binder system, which results in an outstanding hardness of the silica layer. Due to the enormous abrasion resistance the formation of dust on the surface of the ADAMANT is minimised.

The following advantages result:

- The plate can be easily labelled, e.g. with a lead pencil for clear identification
- The abrasion resistance prevents undesired changes or damage to the layer (thickness) during transportation or handling
- A dust-free plate allows convenient handling and avoids silica contamination of the TLC chamber

In addition to the advantages of the optimised binder system the UV indicator of the ADAMANT features an increased brilliance which, in combination with the low-noise background, results in an increased detection sensitivity. Thus ADAMANT is very well suited for trace analyses.

Compared to SIL G or DURASIL plates ADAMANT shows an improved separation efficiency, since the particle size distribution of the silica was optimised.

The selectivity of ADAMANT is different compared to Sil G or DURASIL. Thus the chromatographer now can choose from 3 different types of MN plates in order to optimise a given separation.

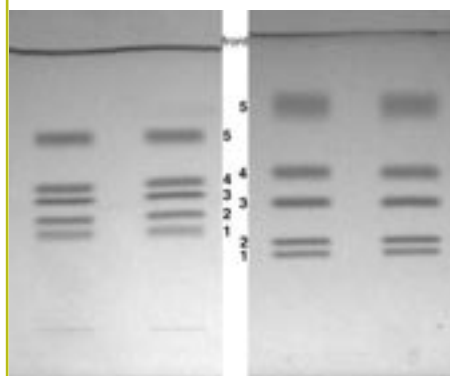
The examples show, that the different selectivities result in different separations under identical conditions and thus allow to improve and optimise critical separations. It should be noted, that the ADAMANT separates the critical pairs of



all shown examples even with a low migration distance of the solvent front. This indicates the high separation efficiency and plate number of the TLC plates.

The low height of the solvent front also improves the bandwidth, as can be seen in the separation of steroids.

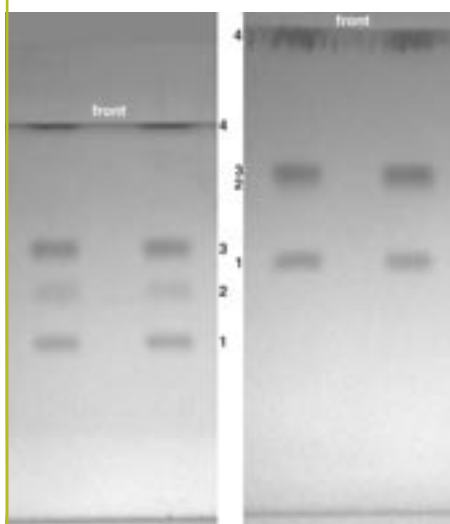
### Separation of steroids



- Peaks:**
1. Cortisone
  2. Corticosterone
  3. Testosterone
  4. Deoxycorticosterone
  5. Progesterone

Layers: left ADAMANT UV<sub>254</sub>, right DURASIL-25 UV<sub>254</sub>  
 Eluent: chloroform / methanol (97:3)  
 Sample: 0.1% in chloroform  
 Migration distance: left 5.0 cm, right 5.3 cm in 10 min

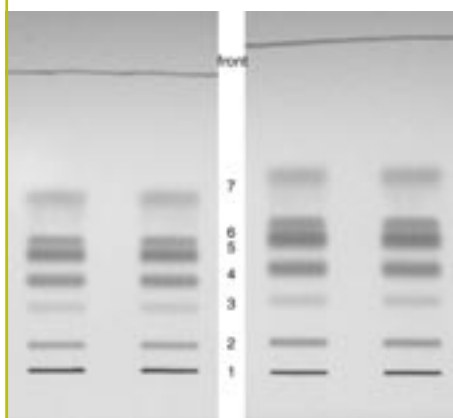
### Separation of flavonoids



- Peaks:**
1. Rutine
  2. Chlorogenic acid
  3. Hyperoside
  4. Quercetine

Layers: left ADAMANT UV<sub>254</sub>, right SIL G-25 UV<sub>254</sub>  
 Eluent: water / formic acid / methyl ethyl ketone / ethyl acetate (1:1:3:5)  
 Sample: 0.1% in methanol  
 Migration distance: left 8.0 cm, right 8.7 cm in 40 min

### Separation of anthraquinone dyes



- Peaks:**
1. Violet 1
  2. Green-blue
  3. Blue 1
  4. Green
  5. Red
  6. Violet 2
  7. Blue 3

Layers: left ADAMANT UV<sub>254</sub>, right DURASIL-25 UV<sub>254</sub>  
 Eluent: toluene / cyclohexane (2:1)  
 Sample: 0.1% in chloroform  
 Migration distance: left 5.4 cm, right 5.8 cm in 10 min



# Ready-to-use layers for TLC

## Standard silica · TLC ready-to-use layers

### Ordering information

Designation	Thickness of layer	Plate size [cm]								
		4 x 8	2.5 x 7.5	5 x 7.5	5 x 10	5 x 20	10 x 10	10 x 20	20 x 20	40 x 20
<b>Silica 60</b>										
specific surface (BET) ~ 500 m <sup>2</sup> /g, mean pore size 60 Å, specific pore volume 0.75 ml/g, particle size 5 – 17 µm										
<b>Glass plates</b>										
SIL G-25	0.25 mm				50/pack	100/pack	25/pack	50/pack	25/pack	
					<b>809017</b>	<b>809011</b>		<b>809012</b>	<b>809013</b>	
SIL G-25 UV <sub>254</sub>	0.25 mm				<b>809027</b>	<b>809021</b>	<b>809020</b>	<b>809022</b>	<b>809023</b>	
SIL G-25 UV <sub>254+366</sub>	0.25 mm					<b>809121</b>		<b>809122</b>	<b>809123</b>	
SIL G-50	0.50 mm									20 Stück
										<b>809051</b>
SIL G-50 UV <sub>254</sub>	0.50 mm									<b>809053</b>
SIL G-100	1.00 mm									15 Stück
										<b>809061</b>
SIL G-100 UV <sub>254</sub>	1.00 mm									<b>809063</b>
SIL G-200	2.00 mm									12 Stück
										<b>809073</b>
SIL G-200 UV <sub>254</sub>	2.00 mm									<b>809083</b>
<b>POLYGRAM® polyester sheets</b>										
SIL G	0.20 mm	50/pack				50/pack			25/pack	25/pack
		<b>805032</b>				<b>805012</b>			<b>805013</b>	<b>805014</b>
SIL G/UV <sub>254</sub>	0.20 mm	<b>805021</b>				<b>805022</b>			<b>805023</b>	<b>805024</b>
SIL G/UV <sub>254</sub>	0.20 mm							Roll 500 x 20 cm		<b>805017</b>
<b>ALUGRAM® aluminium sheets</b>										
SIL G	0.20 mm	50/pack	20/pack	50/pack	50/pack		20/pack	25/pack		
			<b>818030.20</b>	<b>818161</b>	<b>818032</b>		<b>818163</b>	<b>818033</b>		
SIL G/UV <sub>254</sub>	0.20 mm	<b>818131</b>	<b>818130.20</b>	<b>818160</b>	<b>818132</b>		<b>818162</b>	<b>818133</b>		
<b>DURASIL</b>										
silica 60 as above, however, with special binder system										
<b>Glass plates</b>										
DURASIL-25	0.25 mm				50/pack	100/pack		50/pack	25/pack	
					<b>812001</b>	<b>812002</b>		<b>812003</b>	<b>812004</b>	
DURASIL-25 UV <sub>254</sub>	0.25 mm				<b>812005</b>	<b>812006</b>		<b>812007</b>	<b>812008</b>	
<b>ADAMANT</b>										
silica 60 as above, however, with improved binder system and optimised particle size distribution										
<b>Glass plates</b>										
ADAMANT UV <sub>254</sub>	0.25 mm	100/pack			50/pack	100/pack	25/pack	50/pack	25/pack	
		<b>821005</b>			<b>821010</b>	<b>821015</b>	<b>821020</b>	<b>821025</b>	<b>821030</b>	
<b>Silica 60 HR</b>										
high purity silica 60 as above, however, with special binder system and higher gypsum content										
<b>POLYGRAM® polyester sheets</b>										
SIL N-HR	0.20 mm				50/pack			25/pack		
					<b>804012</b>			<b>804013</b>		
SIL N-HR/UV <sub>254</sub>	0.20 mm				<b>804022</b>			<b>804023</b>		
For plates SIL G-HR for aflatoxin separation please see page 286.										



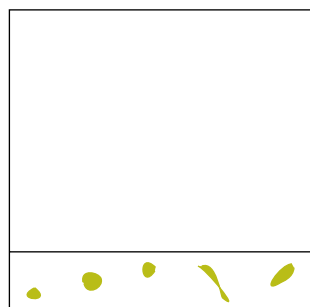


## Standard silica · TLC ready-to-use layers

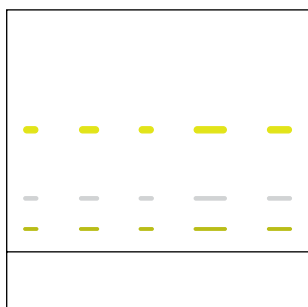
### Concentrating zone · glass plates

Glass plates with concentrating zone are available as SILGUR-25 with standard silica coating and as high performance TLC plate Nano-SILGUR-20.

Silica layers with concentrating zone take advantage of the completely different adsorption properties of two customary



arbitrary application of samples in a concentrating zone



developed chromatogram

chromatographic adsorbents in one plate. Contrary to silica the concentrating zone of kieselguhr is completely inert, with respect to adsorption, towards a large number of compounds.

The sample to be separated is applied to the concentrating zone and developed with a suitable eluent; irrespective of shape, size or position of the spot, the samples always form a narrow band at the interface of the two adsorbents, i.e. kieselguhr and silica. Separation then begins at this point. With identical samples the separated substances always appear at the same height, as illustrated by the figures above.

The concentrating zone serves as a "quick application zone", which allows quantitative evaluation of chromatograms, even if samples have been applied with an unsteady hand. Since careful sample application is time-consuming or requires special equipment, silica plates with concentrating zone are real time-savers. This includes economy in preparation time because of a reduction in evaporation time: larger volumes of dilute solutions can be applied instead of smaller quantities of concentrated solutions. This is often of advantage especially in biochemical investigations.

### Ordering information

Designation	Thickness of layer	Plate size [cm]			Fluorescent indicator
		10 x 10 25 / pack	10 x 20 50 / pack	20 x 20 25 / pack	
<b>SILGUR plates with concentrating zone</b>					
silica 60 with kieselguhr zone for rapid sample application					
<b>Glass plates</b>					
SILGUR-25	0.25 mm		<b>810012</b>	<b>810013</b>	–
SILGUR-25 UV <sub>254</sub>	0.25 mm		<b>810022</b>	<b>810023</b>	UV <sub>254</sub>
<b>Nano-SILGUR plates with concentrating zone</b>					
Nano silica 60 with kieselguhr zone for rapid sample application					
<b>Glass plates</b>					
Nano-SILGUR-20	0.20 mm		<b>811032</b>		–
Nano-SILGUR-20 UV <sub>254</sub>	0.20 mm		<b>811042</b>		UV <sub>254</sub>

## Nano silica · HPTLC ready-to-use layers

In addition to our TLC ready-to-use layers with standard silica coating we manufacture high performance ready-to-use layers under the name "nano silica". We use the same silica 60 which has been successfully applied in thin layer chromatography for about thirty years, however, the adsorbent, as in HPLC, is narrowly fractionated. We use mean particle sizes of 2 – 10 µm, and these allow theoretical plate heights, which are 1 order of magnitude smaller than on standard silica layers.

Advantages of the nano ready-to-use layers compared to standard silica layers are

- **sharper separations** due to small particle size and narrow fractionation. Theoretical plate heights (h values) are considerably smaller than those of the standard TLC plate.

- **shorter developing times** and **shorter migration distances**. After only a few centimeters an optimal separation is achieved.

- **smaller samples** of 0.01 – 0.1 µl (10 – 100 nanoliters). The samples applied are considerably smaller than with standard plates, thus it is possible to apply a large number of samples to a very small surface area, without samples interfering with each other.

- **minimal diffusion** since spots are smaller than 1 mm in diameter.

- **increased detection sensitivity** (nanogram level, hence nano plate). With fluorescence evaluation picogram quantities can be detected.

# Ready-to-use layers for TLC

## Nano silica · HPTLC ready-to-use layers

The layer consists of silica 60 with a mean pore size of 60 Å, specific surface of 500 m<sup>2</sup>/g, a specific pore volume of 0.75 ml/g, but a mean particle size of only 2 – 10 µm. As fluorescent indicator we use manganese activated zinc silicate for short-wave UV light of 254 nm. As binder we add a highly polymeric product, which is stable in almost all organic solvents and towards aggressive visualisation reagents.

### Ready-to-use aluminium sheets ALUGRAM® with nano grade silica

Ready-to-use layers with aluminium support, measuring 5 x 20 or 20 x 20 cm, are available for HPTLC, too. These layers combine the advantages of the nano silica with the advantages of the support aluminium.

### DURASIL glass plates for HPTLC

Similar to the DURASIL plates with standard silica we also produce HPTLC plates Nano-DURASIL with nano silica (mean pore diameter 60 Å, pore volume about 0.75 ml/g, specific surface 500 m<sup>2</sup>/g, particle size 2–10 µm). Nano-DURASIL plates differ from the well-known plates Nano-SIL G-20 by the binder system, which results in hard, water-resistant and wettable layers with outstanding separation properties. Nano-DURASIL plates, too, are available with or without fluorescent indicator (green fluorescence, 254 nm).

If one compares the performance of the Nano-DURASIL-20 plates with the alternative Nano-SIL G-20 plates for the separation of anthraquinone dyes (see figure), one observes a similar selectivity, with an even better separation result for Nano-DURASIL-20 as compared to Nano-SIL G-20.

Differences in the characteristics of DURASIL plates compared to conventional silica plates are described in detail in the chapter "Standard silica layers" on page 266.

Nano-DURASIL-20 plates show superior separation characteristics in the fields of environmental, clinical, medical and organic analyses. Substances separated so far include flavonoids, barbiturates, bile acids, nitroanilines, pesticides, and steroids.

### Ordering information

Designation	Thick-ness of layer	Plate size [cm]				Fluorescent indicator
		5 x 5 100 / pack	5 x 20 50 / pack	10 x 10 25 / pack	10 x 20 50 / pack	
<b>Nano silica</b> silica 60, specific surface (BET) ~ 500 m <sup>2</sup> /g, mean pore size 60 Å, specific pore volume 0.75 ml/g, particle size 2 – 10 µm						
<b>Glass plates</b>						
Nano-SIL-20	0.20 mm	<b>811011</b>		<b>811012</b>	<b>811013</b>	–
Nano-SIL-20 UV <sub>254</sub>	0.20 mm	<b>811021</b>		<b>811022</b>	<b>811023</b>	UV <sub>254</sub>
<b>ALUGRAM® aluminium sheets</b>						
Nano-SIL G	0.20 mm		<b>818140</b>		<b>818141</b>	–
Nano-SIL G/UV <sub>254</sub>	0.20 mm		<b>818142</b>		<b>818143</b>	UV <sub>254</sub>
<b>Nano-DURASIL</b> Nano silica as above, however, with special binder system						
<b>Glas plates</b>						
Nano-DURASIL-20	0.20 mm	<b>812009</b>		<b>812010</b>	<b>812011</b>	–
Nano-DURASIL-20 UV <sub>254</sub>	0.20 mm	<b>812012</b>		<b>812013</b>	<b>812014</b>	UV <sub>254</sub>

For more applications see our application database on the internet.

### Comparison of TLC and HPTLC plates

#### Separation of anthraquinone dyes

Layers A: a) SIL G-25 UV<sub>254</sub>, b) DURASIL-25 UV<sub>254</sub>

Layers B: a) Nano-SIL G-20 UV<sub>254</sub>  
b) Nano-DURASIL-20 UV<sub>254</sub>

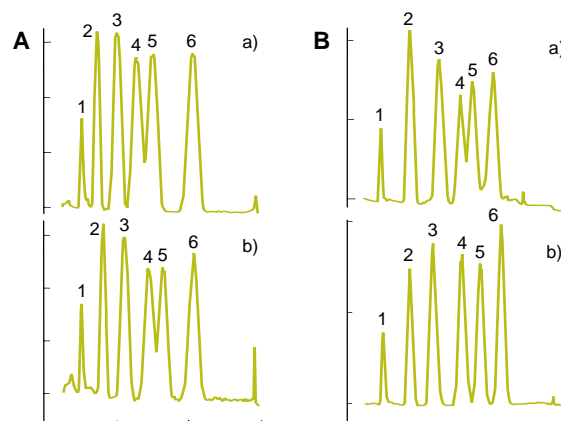
Eluent: Toluol/Cyclohexan (2:1, v/v)

Migration distance: A: 4.8 cm in 8 min  
B: a) 4.3 cm in 8 min, b) 4.7 cm in 8 min

Detection: TLC scanner, UV 254 nm

#### Peaks :

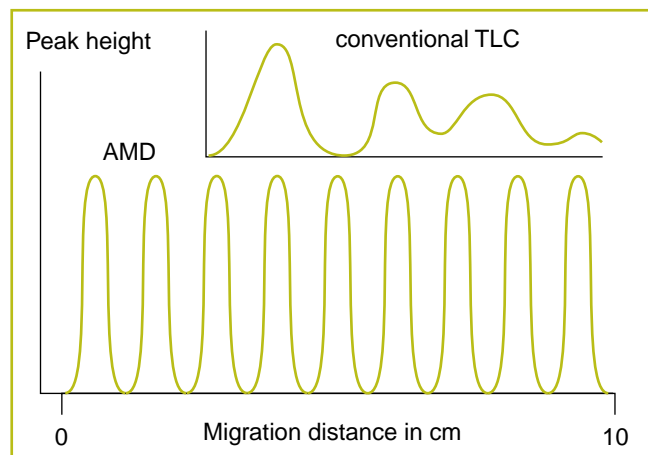
1) blue 3, 2) violet 2, 3) red, 4) green, 5) blue 1, 6) violet 1





## Nano silica · HPTLC ready-to-use layers

### AMD plates



Nano plates with extremely thin layers of silica 60 (0.05 or 0.1 mm thick) can be used for conventional HPTLC, but are especially suited for the AMD procedure (AMD = Automated Multiple Development), which is explained in this chapter.

Rapid and efficient analyses at ultra trace levels require methods, which allow simultaneous investigation of several active ingredients in a large number of samples. Such multi-methods considerably increase the sample throughput and thus lower the cost per analysis. The TLC / AMD procedure, which has been developed by the Central Analytical Department of the Bayer AG, Dormagen, Germany, allows the simultaneous determination of 40 plant protective chemicals from 14 extracts on one TLC plate <sup>1)</sup>.

#### Principle:

Compared to conventional multiple development of TLC plates, in which the total migration distance is passed several times, an AMD development is performed in many partial steps of different length, the number of individual runs being between 10 and 30. The migration distance of each step is, by a defined increment, longer than the previous step. In general, these increments are between 1 and 3 mm. Between all steps, the TLC plate is dried in vacuum <sup>1)</sup>.

#### Procedure:

After application of the samples, the AMD process is started by evacuation of the chromatographic tank, in order to remove residual solvent of the standard or sample solutions from the silica layer. Next, the chromatographic tank is ventilated with an inert atmosphere. The tank is charged with the developing solvent, and the first run is started. After the extremely short developing time of a few seconds the mobile phase is again removed by evacuation. Then the next cycle is started with ventilation, a somewhat longer developing time (see above) and another removal of mobile phase in vacuum.

With isocratic multiple development, the separation efficiency is significantly higher, because the total migration distance of the solvent front over all individual runs is in the range of a meter, and this large distance is passed in the first, fast-running centimetres of the plate <sup>2)–5)</sup>.

Additionally, the detection of substances is improved compared to conventional TLC, because in every cycle the substance zones are passed by the solvent front, and thus focussed. Consequently, the peak width is independent of the migration distance and the width of the applied spots. This allows application of larger sample volumes.

The AMD procedure also allows gradient elution on silica, if the tank is filled with a solvent of decreasing polarity for every run <sup>1)</sup>.

For production of the nano plates with extremely thin layer we use the same nano silica 60 with mean pore size of 60 Å, a specific surface of 500 m<sup>2</sup>/g, a specific pore volume of 0.75 ml/g and a mean particle size of only 2 – 10 µm. A manganese-activated zinc silicate for short-wave UV light of 254 nm is used as fluorescent indicator. The binder is a highly polymeric product, which is resistant in almost all organic solvents and towards many aggressive detection reagents. The support of the plate is glass.

#### References

- 1) K. Burger, Pflanzenschutz-Nachrichten-Bayer **41,2** (1988) 173
- 2) J. A. Perry, K. W. Haag and L. J. Glunz, J. Chromatographic Science **11** (1973) 447
- 3) T. H. Jupille, J. A. Perry, J. Chromatography **99** (1974) 231
- 4) T. H. Jupille and J. A. Perry, Science **194** (1976) 288
- 5) K. Burger, Fresenius Z. Anal. Chem. **318** (1984) 228

### Ordering information

Designation	Thickness of layer	Plate size	Fluorescent indicator
		10 x 20 cm	
<b>Nano silica</b> silica 60, specific surface (BET) ~ 500 m <sup>2</sup> /g, mean pore size 60 Å, specific pore volume 0.75 ml/g, particle size 2 – 10 µm			
<b>Glass plates with extremely thin nano silica layer</b>			
AMD SIL G-05 UV <sub>254</sub>	0.05 mm	5 / pack <b>811101</b>	UV <sub>254</sub>
AMD SIL G-10 UV <sub>254</sub>	0.10 mm	25 / pack <b>811103</b>	UV <sub>254</sub>

# Ready-to-use layers for TLC

## TLC and HPTLC plates with modified silica layers

### RP plates · HPTLC ready-to-use layers with C<sub>18</sub> silica

TLC ready-to-use layers coated with hydrophilic silica have been in use for a number of years, and the majority of all TLC separation problems can be solved with these layers.

However, it can be advantageous to make certain separations on lipophilic silica layers, i.e. in reversed phase. Due to the rapid development of HPLC there is an increasing demand for TLC plates which can be used for orienting pre-separations. In HPLC frequently reversed phase systems based on C<sub>8</sub> or C<sub>18</sub> silanized silicas are used. These systems are especially suited for the separation of very polar (hydrophilic) classes of compounds, which previously could only be separated by ion exchange chromatography. RPTLC with alkyl silanised silicas as stationary phases considerably broadens the applicability of TLC.

### HPTLC plates Nano-SIL C 18-50 and Nano-SIL C 18-100

After careful consideration we have decided, that instead of the different alkyl silanes used in HPLC (e.g. C<sub>2</sub>, C<sub>4</sub>, C<sub>8</sub> or C<sub>18</sub>) we use only C<sub>18</sub> silane for our reversed phase TLC plates, but with different percentages of silanisation. Practical experience has confirmed the validity of our considerations. For a dimethyl modified silica layer see page 274.

Our RP plates are produced using a medium pore size silica (mean pore diameter 60 Å), with a mean particle size of 2 – 10 µm (identical to the material used for our nano silica plates). This nano silica is reacted to a different degree, either totally (= 100%) or partially (= 50% of the reactive groups). Thus we meet a broad range of applications: anhydrous eluents as well as eluents with high concentrations of water can be used (see table).

As fluorescent indicator we use an acid-resistant product which allows application of strongly acidic eluents without undesirable fluorescence quenching. This fluorescence indicator shows a pale blue fluorescence in short-wave UV light. UV absorbing substances appear as dark-blue to black spots on a light-blue background.

The two available layers differ by their selectivity, possibly due to a combination of hydrophobic and polar interactions. Furthermore, of course they show different wettability (hydrophobicity). The table shows migration distances for different contents of water.

### Ordering information

Designation	Thickness of layer	Plate size 10 x 10 cm 25 / pack	Fluorescent indicator
<b>Nano-SIL C 18</b>			
Nano silica with octadecyl modification (RP-18), degree of silanisation 100 % for C 18-100, 50 % for C 18-50			
<b>Glass plates</b>			
Nano-SIL C 18-50	} 50 % silanised	0.20 mm	<b>811054</b>
Nano-SIL C 18-50 UV <sub>254</sub>		0.20 mm	<b>811064</b>
Nano-SIL C 18-100	} 100 % silanised	0.20 mm	<b>811052</b>
Nano-SIL C 18-100 UV <sub>254</sub>		0.20 mm	<b>811062</b>
			– UV <sub>254</sub> – UV <sub>254</sub>

Substances separated on Nano-SIL C 18-50 and Nano-SIL C 18-100 include the following classes:

- alkaloids
- amino acids
- preservatives
- optical brighteners
- barbiturates
- polycyclic aromatic hydrocarbons (PAHs)
- drugs
- peptides
- flavonoids
- phenols
- indole derivatives
- steroids

If your separation requires a wettable layer, we recommend our TLC plate RP-18 W/UV<sub>254</sub>, which is described in the following paragraph.

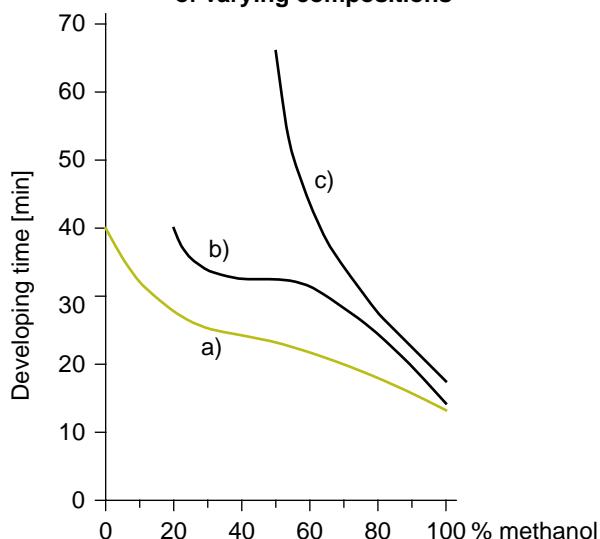
Migration of C 18-50 and C 18-100 silica layers as compared to RP-18 W/UV <sub>254</sub> plates				
Eluent	v/v	Migration distances [mm / 15 min]		
		C 18-50	C 18-100	RP-18 W
methanol/H <sub>2</sub> O	2:1	57	45	44
	1:1	52	21	40
	1:2	50	0	43
	1:3	40	0	45
	1:4	30	0	46
	0:1	0	0	54
acetonitrile/H <sub>2</sub> O	2:1	62	46	66
	1:1	52	30	54
	1:2	51	27	46
	1:3	48	15	44
	1:9	20	0	42
chloroform		68	64	71



## TLC and HPTLC plates with modified silica layers

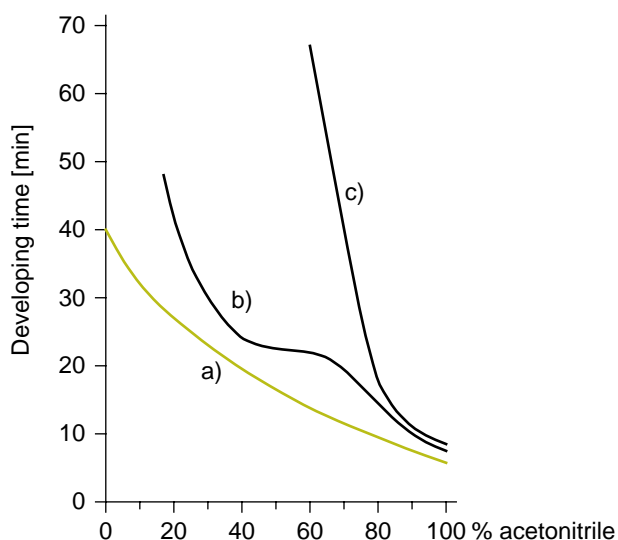
### RP plates · HPTLC ready-to-use layers with C<sub>18</sub> silica

Elution properties of different RP plates in mixtures of methanol/water and acetonitrile/water of varying compositions



Developing time for a migration distance of 7 cm versus eluent composition for the system methanol/water on different RP plates:

- a) RP-18 W/UV<sub>254</sub>    b) Nano-SIL C 18-50 UV<sub>254</sub>  
c) Nano-SIL C 18-100 UV<sub>254</sub>



Developing time for a migration distance of 7 cm versus eluent composition for the system acetonitrile/water on different RP plates:

- a) RP-18 W/UV<sub>254</sub>    b) Nano-SIL C 18-50 UV<sub>254</sub>  
c) Nano-SIL C 18-100 UV<sub>254</sub>

### RP-18 W/UV<sub>254</sub> · a wettable TLC plate for reversed phase and normal phase chromatography

The TLC glass plates RP-18 W/UV<sub>254</sub> are "hybrid" plates for reversed phase (RP) as well as for normal phase (NP) chromatography. Formerly, TLC plates were either reversed phase plates with totally hydrophobic layer, e.g. Nano-SIL C 18-100, or normal phase plates with totally hydrophilic layer, e.g. SIL G-25.

TLC plates RP-18 W/UV<sub>254</sub> can be developed with purely organic and organic/aqueous solvents as well as with purely aqueous eluents. A partially silanised C<sub>18</sub> silica with defined number of residual silanol groups allows a more "RP type" or "NP type" separation depending on eluent composition. Thus the polarity of the layer can be determined by the relative polarity of the eluent. These plates can be used with acid eluents as well, because the layer contains an acid-resistant fluorescent indicator. The mean particle size of the silica is 9 µm.

So far the following classes of compounds could be separated successfully on RP-18 W/UV<sub>254</sub>:

- aminophenols
- barbiturates
- preservatives
- nucleobases
- polycyclic aromatic hydrocarbons
- steroids
- tetracyclines
- plasticizers (phthalates)

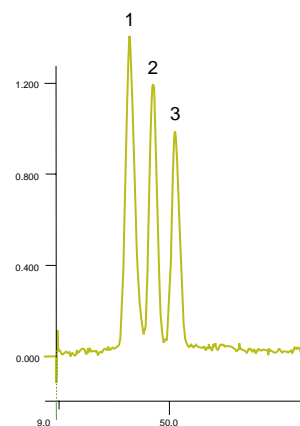
Activation of the layer at 110 – 115 °C for 10 – 15 min often improves the separation..

### Separation of salicyl derivatives

Layer: ALUGRAM® RP-18 W/UV<sub>254</sub>  
Sample volume: 250 nl  
Eluent: methanol / 1 N acetic acid (25:75, v/v)  
Migration distance: 9 cm in 30 min  
Detection: TLC scanner, UV 254 nm

#### Peaks:

1. Salicylic acid (1.0%)
2. Acetylsalicylic acid (1.0%)
3. Salicyluric acid (0.4%)



# Ready-to-use layers for TLC

## TLC and HPTLC plates with modified silica layers

### RP plates · HPTLC ready-to-use layers with C<sub>18</sub> silica

#### ALUGRAM® RP-18 W/UV<sub>254</sub> · the aluminium sheet for reversed phase HPTLC

This reversed phase ready-to-use aluminium sheets combine the advantages of our outstanding RP-18 W/UV<sub>254</sub> layer with those of the flexible support aluminium. Aluminium is temperature-resistant like glass, but features the advantages

of being nonbreakable, requiring less packaging and lower storage space. The outstanding adherence of the layer on the support allows cutting of any size, simply with scissors, or cutting out interesting components after separation, making them available for subsequent investigations (e.g. IR, MS).

#### Ordering information

Designation	Thickness of layer	Plate size [cm]						Fluorescent indicator
		4 x 8	5 x 10	5 x 20	10 x 10	10 x 20	20 x 20	
<b>RP-18 W/UV<sub>254</sub></b>								
silica with partial octadecyl modification (C 18), wettable with water								
<b>Glass plates</b>								
RP-18 W/UV <sub>254</sub>	0.25 mm			50 / pack <b>811073</b>	25 / pack <b>811075</b>	50 / pack <b>811072</b>	25 / pack <b>811071</b>	UV <sub>254</sub>
RP-18 W/UV <sub>254</sub>	1.00 mm						15 / pack <b>811074</b>	UV <sub>254</sub>
<b>ALUGRAM® aluminium sheets</b>								
RP-18 W/UV <sub>254</sub>	0,15 mm	50 / pack <b>818144</b>	50 Stück <b>818152</b>	50 / pack <b>818145</b>	25 / pack <b>818147</b>		25 / pack <b>818146</b>	UV <sub>254</sub>

### RP plates · RP-2/UV<sub>254</sub> – silanised silica layer for TLC

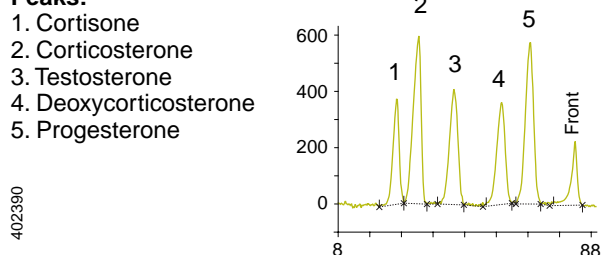
In pharmacopoeias the RP-2 phase is called "silanised silica", i.e. like in HPLC and SPE the term "2" stands for dimethyl and should not be confused with ethyl. The silica used for production has a pore size of 60 Å and a particle size distribution between 5 and 17 µm. The layer contains an acid-resistant indicator with a blue fluorescence at 254 nm. The TLC plate RP-2/UV<sub>254</sub> can be developed with purely organic, organic/aqueous and purely aqueous eluents. Thus the phase finds numerous applications in normal phase chromatography as well as in the reversed phase mode. Applications include the analysis of active plant constituents and steroids.

#### Separation of steroids

Layer: RP-2/UV<sub>254</sub>  
 Eluent: dichloromethane / methanol (98.5 : 1.5)  
 Detection: TLC scanner, UV 254 nm

#### Peaks:

1. Cortisone
2. Corticosterone
3. Testosterone
4. Deoxycorticosterone
5. Progesterone



#### Ordering information

Designation	Thickness of layer	Plate size [cm]				Fluorescent indicator	
		4 x 8	5 x 10	10 x 20	20 x 20		
<b>RP-2/UV<sub>254</sub></b>							
"silanised silica" = dimethyl-modified silica 60							
<b>Glass plates</b>							
RP-2/UV <sub>254</sub>	0.25 mm			10 x 10 25 / pack	10 x 20 50 / pack	20 x 20 25 / pack	UV <sub>254</sub>
<b>ALUGRAM® aluminium sheets</b>							
RP-2/UV <sub>254</sub>	0.15 mm	<b>818170</b>				<b>818171</b>	UV <sub>254</sub>



## TLC and HPTLC plates with modified silica layers

### Nano-SIL CN – the cyano-modified ready-to-use layer

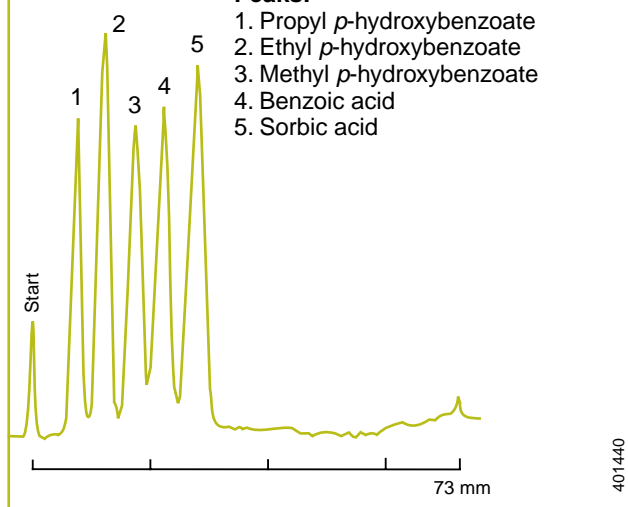
#### Separation of preservatives

Layer: Nano-SIL CN/UV  
 Sample volume: 400 nl  
 Eluent: ethanol / water / glacial acetic acid  
 20:80:0.2 with 0.1 mol/l  
 tetraethylammonium chloride

Migration distance: 7.3 cm in 30 min  
 Detection: TLC scanner, UV 254 nm

#### Peaks:

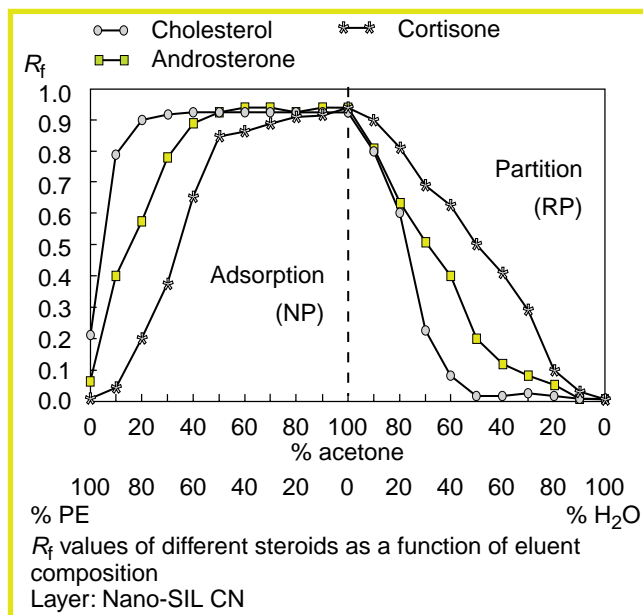
1. Propyl *p*-hydroxybenzoate
2. Ethyl *p*-hydroxybenzoate
3. Methyl *p*-hydroxybenzoate
4. Benzoic acid
5. Sorbic acid



For manufacturing this plate, again nano grade silica is used. The cyano modification results in plates with a somewhat more hydrophobic character compared to the amino plate, with the following series of polarities: silica > DIOL > NH<sub>2</sub> > CN > RP-2 > C 18-50 > RP-18 W > C 18-100. As with our other plates, we use an acid-resistant indicator with a blue fluorescence at 254 nm. This neutral ready-to-use layer can be wetted equally well with water as with organic solvents, thus the plate can be used with all conventional TLC eluents.

Similar to the RP-18 W plate, Nano-SIL CN shows normal phase or reversed phase separation modes depending on the polarity of the developing solvent.

The following figure illustrates the transition from the NP character to the RP character for the example of steroids, in which the polarity of the eluent governs the type of separation mechanism. If the separation is performed in pure petroleum ether (PE), the steroids are strongly retained due to adsorptive interactions with the active sites of the stationary phase. Logically, this effect is strongest for cortisone with its functional groups. In the eluent system PE – acetone the retention of the steroids decreases with increasing acetone content. When the eluent consists of 50% acetone, all three substances are found more or less in the range of the solvent front. When changing the eluent system to acetone – water, the RP character of the Nano-SIL CN plate becomes evident. First of all the sequence of elution is reversed: the non-polar cholesterol is now more strongly retained than the more polar components. The presence of water increases the retention of the three steroids. In addition to steroid hormones, also phenols and preservatives can be separated on Nano-SIL CN. For detailed applications using the plates Nano-SIL CN please see our applications database on the internet.



### Ordering information

Designation	Thickness of layer	Plate size [cm]			Fluorescent indicator
		4 x 8 50 / pack	10 x 10 25 / pack	10 x 20 25 / pack	
<b>Nano-SIL CN</b> cyano-modified nano silica					
<b>Glass plates</b>					
Nano-SIL CN/UV	0.20 mm		<b>811115</b>	<b>811116</b>	UV <sub>254</sub>
<b>ALUGRAM® aluminium sheets</b>					
Nano-SIL CN/UV	0.15 mm	<b>818184</b>		<b>818185</b>	UV <sub>254</sub>

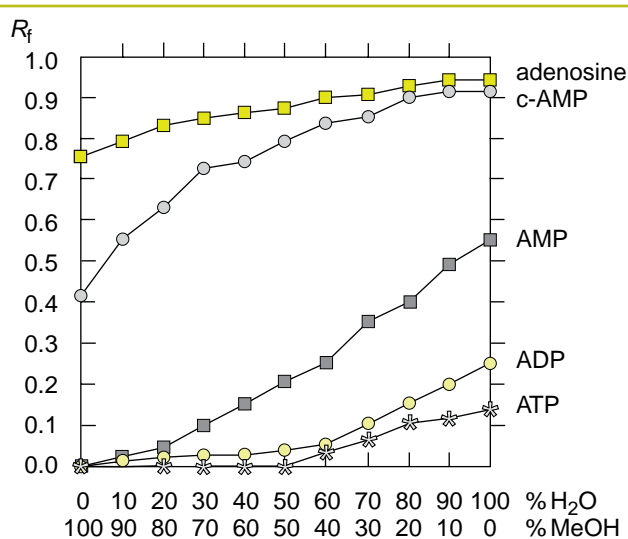
# Ready-to-use layers for TLC

## TLC and HPTLC plates with modified silica layers

### Nano-SIL NH<sub>2</sub> – the amino-modified ready-to-use layer

The base material for this hydrophilic TLC ready-to-use plate is silica with a mean pore size of 60 Å and a mean particle size of 2 – 10 µm, which is identical with the material used for our nano silica plates. The amino modification results in a neutral ready-to-use layer, which is wetted equally well by pure water as by organic solvents. Thus all conventional eluents used in chromatography can be applied without reservation. This layer is available with or without an acid-resistant fluorescent indicator.

The Nano-SIL NH<sub>2</sub> plate is well suited for the separation of vitamins, sugars, steroids, purine derivatives, xanthenes, phenols, nucleotides, and pesticides.



Separation of adenosine, adenosine monophosphate (c-AMP, AMP), adenosine diphosphate (ADP) and adenosine triphosphate (ATP)  
 Layer: Nano-SIL NH<sub>2</sub>/UV  
 Eluent: MeOH/H<sub>2</sub>O according to fig. + 0.18 M NaCl  
 Migration distance 7 cm

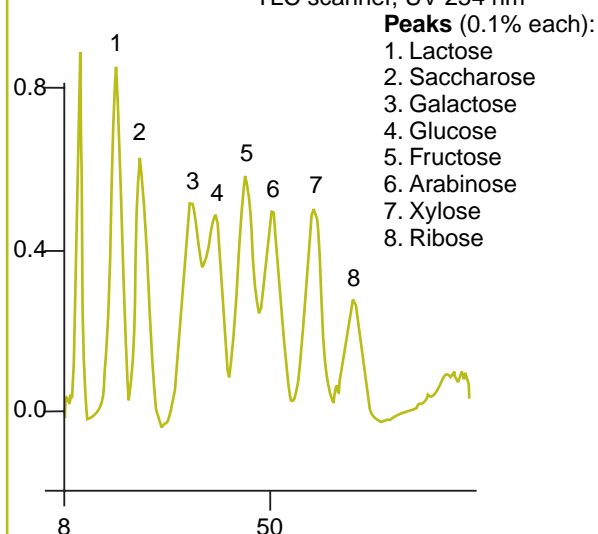
### Separation of sugars

Layer: Nano-SIL NH<sub>2</sub>/UV  
 Eluent: ethyl acetate / pyridine / water / glacial acetic acid (60:30:10:5, v/v/v/v)

Migration distance: 8 cm in 45 min, double development

Sample volume: 500 nl

Detection: dry layer at 160 °C for 5 min, TLC scanner, UV 254 nm



For detailed applications using the plates Nano-SIL NH<sub>2</sub> please see our applications database on the internet.

[www.mn-net.com](http://www.mn-net.com)

### Ordering information

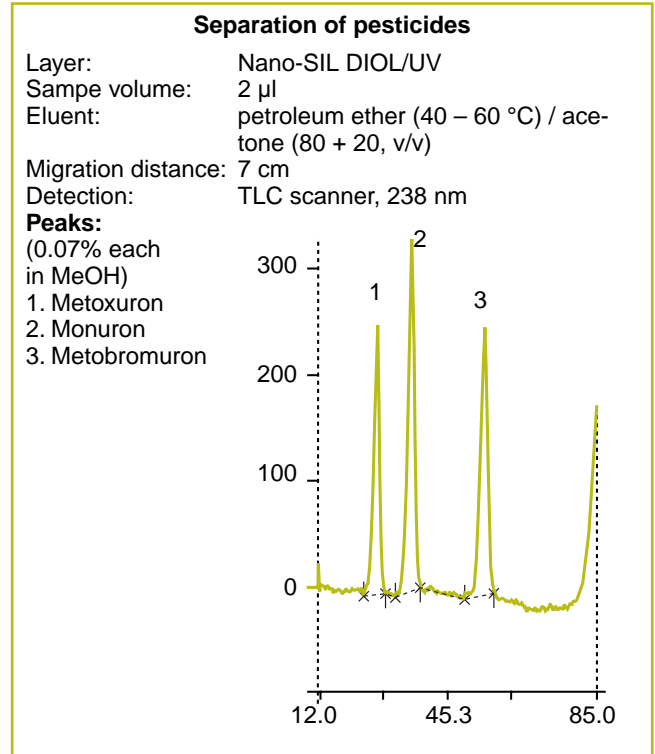
Designation	Thickness of layer	Plate size [cm]			Fluorescent indicator
		4 x 8 50 / pack	10 x 10 25 / pack	10 x 20 25 / pack	
<b>Nano-SIL NH<sub>2</sub></b>					
amino-modified nano silica					
<b>Glass plates</b>					
Nano-SIL NH <sub>2</sub>	0.20 mm		<b>811109</b>	<b>811110</b>	–
Nano-SIL NH <sub>2</sub> /UV	0.20 mm		<b>811111</b>	<b>811112</b>	UV <sub>254</sub>
<b>ALUGRAM® aluminium sheets</b>					
Nano-SIL NH <sub>2</sub> /UV	0.15 mm	<b>818182</b>		<b>818183</b>	UV <sub>254</sub>



## TLC and HPTLC plates with modified silica layers

### Nano-SIL DIOL/UV – the diol modified layer for TLC

For production of this hydrophilic ready-to-use layers we use a nano-grade silica (2 – 10 µm) with a mean pore size of 60 Å. The polarity of this phase is between that of silica and the NH<sub>2</sub> phase. As usual the layer contains the acid-resistance indicator with a blue fluorescence at 254 nm. The DIOL phase, too, can be used with water or with organic solvents and can thus be used with all common TLC eluents. Depending on the conditions this plates can be operated in the reversed phase or the normal phase mode. Applications include e. g. steroids, pesticides or plant constituents. Since it is less sensitive to the water content of the environment, the DIOL phase can be used as an alternative to silica in critical separation problems.



### Ordering information

Designation	Thickness of layer	plate size [cm]			Fluorescent indicator
		4 x 8 50 / pack	10 x 10 25 / pack	10 x 20 25 / pack	
<b>Nano-SIL DIOL/UV</b>					
diol-modified nano silica					
<b>Glass plates</b>					
Nano-SIL DIOL/UV	0.20 mm		<b>811120</b>	<b>811121</b>	UV <sub>254</sub>
<b>ALUGRAM® aluminium sheets</b>					
Nano-SIL DIOL/UV	0.15 mm	<b>818180</b>		<b>818181</b>	UV <sub>254</sub>

### HPTLC method development kits

To facilitate selection of the optimum HPTLC plate for a given separation, we offer HPTLC method development kits.

Composition	Cat. No.
<b>Development kit with glass plates</b>	
3 glass plates 10 x 10 cm (scored to 5 x 10 cm) each of Nano-SIL C18-100/UV <sub>254</sub> , RP-18 W/UV <sub>254</sub> , RP-2/UV <sub>254</sub> , Nano-SIL CN/UV, Nano-SIL NH <sub>2</sub> /UV, Nano-SIL DIOL/UV	<b>811001</b>
<b>Development kit with ALUGRAM® aluminium sheets</b>	
5 sheets 4 x 8 cm each of RP-18 W/UV <sub>254</sub> , RP-2/UV <sub>254</sub> , Nano-SIL CN/UV, Nano-SIL NH <sub>2</sub> /UV, Nano-SIL DIOL/UV	<b>818001</b>

# Ready-to-use layers for TLC

## Aluminium oxide - TLC ready-to-use layers

For general information on aluminium oxide as adsorbent in TLC please refer to the chapter "Adsorbents in TLC" on page 296. Our TLC ready-to-use layers are coated with basic aluminium oxide (about pH 9). To ensure sufficient adherence an inert organic binder is added to the aqueous suspension for coating. The aluminium oxide has a specific surface of about 200 m<sup>2</sup>/g (BET) and a pore size of about 60 Å.

**We recommend to activate aluminium oxide ready-to-use layers before use by heating 10 minutes at 120 °C**

### Separation of bisadducts of fullerenes

F. Djojo, A. Hirsch, Chem. Eur. J. **4** (1998), 344 – 356

Layer: ALUGRAM® ALOX N/UV<sub>254</sub>,  
20 x 20 cm, Cat. No. 818 023

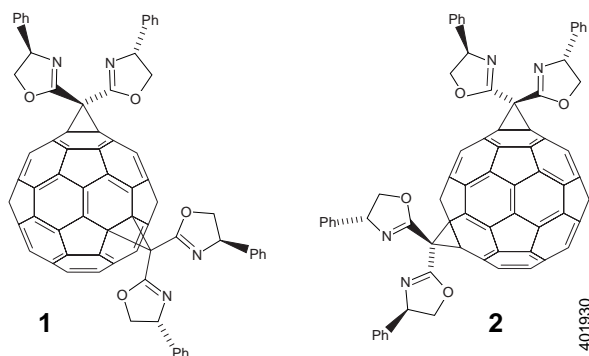
Eluent: toluene/ ethyl acetate (95:5, v/v)

Detection: UV, 254 nm

R<sub>f</sub> values:

Bis[bis(4-phenyloxazolin)methan]fullerene 1: 0.14

Bis[bis(4-phenyloxazolin)methan]fullerene 2: 0.26



### Separation of lipophilic dyes

Layer: ALOX-25 UV<sub>254</sub>

Sample volume: 1000 nl

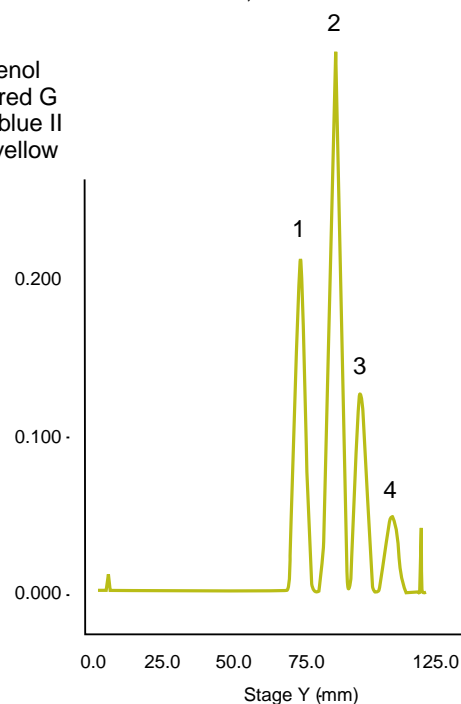
Eluent: toluene/cyclohexane (2:1, v/v)

Migration distance: 10.8 cm in 15 min

Detection: TLC acanner, UV 254 nm

#### Peaks:

1. Indophenol
2. Sudan red G
3. Sudan blue II
4. Butter yellow



## Ordering information

Designation	Thick-ness of layer	4 x 8	5 x 20	20 x 20	Fluores-cent in-dicator
-------------	---------------------	-------	--------	---------	-------------------------

### Aluminium oxide basic

pH ~ 9, specific surface (BET) ~ 200 m<sup>2</sup>/g, pore size ~ 60 Å, inert organic binder

#### Glass plates

ALOX-25	0.25 mm	100 / pack	25 / pack	–
ALOX-25 UV <sub>254</sub>	0.25 mm	<b>807011</b>	<b>807013</b>	UV <sub>254</sub>
			<b>807023</b>	
ALOX-100 UV <sub>254</sub>	1.00 mm		15 / pack	–
			<b>807033</b>	UV <sub>254</sub>

#### POLYGRAM® polyester sheets

ALOX N	0.20 mm	50 / pack	50 / pack	25 / pack	–
ALOX N/UV <sub>254</sub>	0.20 mm	<b>802012</b>	<b>802013</b>	<b>802013</b>	UV <sub>254</sub>
		<b>802021</b>	<b>802022</b>	<b>802023</b>	

#### ALUGRAM® aluminium sheets

ALOX N	0.20 mm		50 / pack	25 / pack	–
ALOX N/UV <sub>254</sub>	0.20 mm		<b>818025</b>	<b>818013</b>	UV <sub>254</sub>
			<b>818024</b>	<b>818023</b>	



## Cellulose · TLC ready-to-use layers

### Unmodified cellulose layers

Cellulose is especially used for partition chromatographic separations of polar substances such as amino acids and other carboxylic acids or carbohydrates. Compared to paper chromatography (PC) thin layer chromatography on cellulose offers shorter migration times and more concentrated substance spots resulting in a higher detection sensitivity.

TLC ready-to-use layers **CEL 300** are coated with **native, fibrous cellulose**. TLC ready-to-use layers **CEL 400** are coated with **microcrystalline cellulose AVICEL®**. This type of cellulose is prepared by hydrolysis of high purity cellulose with hydrochloric acid. Depending on the starting cellulose and the following clean-up one obtains cellulose crystallites with a mean degree of polymerisation between 40 and 200. The microcrystalline structure of this cellulose can be con-

firmed by X-ray investigations. So far the following compounds could be successfully separated: carboxylic acids, lower alcohols, urea and purine derivatives.

The separation characteristics of CEL 300 and CEL 400 are somewhat different. For this reason we recommend that you try both types for method development and then select the more suitable type from experience.

The fluorescent indicator used for cellulose layers allows in many cases viewing of substances in short-wave UV light of 254 nm. The greenish fluorescence is quenched by substances with a native absorption above about 230 nm; these appear as dark spots. For detection of amino acids or peptides with ninhydrin cellulose ready-to-use layers without fluorescent indicator are better suited than fluorescent layers.

### Ordering information

Designation	Thickness of layer	Plate size [cm]				Fluorescent indicator
		4 x 8	5 x 20	10 x 20	20 x 20	
<b>Cellulose MN 300</b> native fibrous cellulose						
<b>Glass plates</b>						
CEL 300-10	0.10 mm		100 / pack <b>808011</b>	50 / pack <b>808012</b>	25 / pack <b>808013</b>	–
CEL 300-10 UV <sub>254</sub>	0.10 mm		<b>808021</b>	<b>808022</b>	<b>808023</b>	UV <sub>254</sub>
CEL 300-25	0.25 mm			<b>808032</b>	<b>808033</b>	–
CEL 300-25 UV <sub>254</sub>	0.25 mm			<b>808042</b>	<b>808043</b>	UV <sub>254</sub>
CEL 300-50	0.50 mm				<b>808053</b>	–
CEL 300-50 UV <sub>254</sub>	0.50 mm				<b>808063</b>	UV <sub>254</sub>
<b>POLYGRAM® polyester sheets</b>						
CEL 300	0.10 mm	50 / pack <b>801011</b>	50 / pack <b>801012</b>		25 / pack <b>801013</b>	–
CEL 300 UV <sub>254</sub>	0.10 mm		<b>801022</b>		<b>801023</b>	UV <sub>254</sub>
<b>ALUGRAM® aluminium sheets</b>						
CEL 300	0.10 mm	50 / pack <b>818155</b>	50 / pack <b>818154</b>		25 / pack <b>818153</b>	–
CEL 300 UV <sub>254</sub>	0.10 mm		<b>818157</b>		<b>818156</b>	UV <sub>254</sub>
<b>Cellulose MN 400 (AVICEL®)</b> microcrystalline cellulose						
<b>Glass plates</b>						
CEL 400-10	0.10 mm			50 / pack <b>808072</b>	25 / pack <b>808073</b>	–
CEL 400-10 UV <sub>254</sub>	0.10 mm			<b>808082</b>	<b>808083</b>	UV <sub>254</sub>
<b>POLYGRAM® polyester sheets</b>						
CEL 400	0.10 mm		50 / pack <b>801112</b>		25 / pack <b>801113</b>	–
CEL 400 UV <sub>254</sub>	0.10 mm		<b>801122</b>		<b>801123</b>	UV <sub>254</sub>

### Cellulose ion exchangers

Cellulose ion exchange layers are suited for the separation of substances with exchange-active groups. Consequently they are used for the separation of amino acids, peptides, enzymes, nucleic acid constituents (nucleotides, nucleosides) etc. We supply TLC ready-to-use anion exchange layers of

**DEAE cellulose** (diethylaminoethyl cellulose, R-O-C<sub>2</sub>H<sub>4</sub>-N(C<sub>2</sub>H<sub>5</sub>)<sub>2</sub>) and **PEI cellulose**. The latter is not a chemically modified cellulose, but a complex of cellulose and polyethyleneimine. PEI cellulose is a relatively strong anion exchanger, which is mainly used for the analyses of nucleic

# Ready-to-use layers for TLC

## Cellulose · TLC ready-to-use layers

acids. PEI layers should be stored in a refrigerator at 4 °C. When stored at room temperature PEI cellulose tends to show a more or less pronounced yellow colouration, which does not interfere with the separation but can impede the identification of separated substances. Predevelopment with distilled water diminishes an eventual yellow colouration. An important application of POLYGRAM® CEL 300 PEI is the analysis of mutagenic substances with a <sup>32</sup>P postlabelling procedure (see application).

### Mixed layers of cellulose DEAE and HR for separation of mono- and oligonucleotides in nucleic acid hydrolyzates

The Medical Research Council Laboratory of Molecular Biology in Cambridge (UK) has developed a special procedure for the separation of radioactively labelled mono- and oligonucleotides in hydrolyzates of ribonucleic acid. It is a 2-dimensional procedure, in which mononucleotides and oligonucleotides are separated up to n = 50. The separation process consists of 2 stages, first a high voltage electrophoretic group fractionation on acetate sheets in the 1st dimension and then a thin layer chromatographic separation in the 2nd dimension after blotting of the pre-separated substances onto a mixed layer of DEAE cellulose and HR cellulose in the ratio 2 : 15. As eluent concentrated urea solutions with addition of homomix solutions are used, which consist of ribonucleic acid hydrolyzates and dialyzates. Mononucleotides move up to the front, and depending on chain length the oligonucleotides appear between the R<sub>f</sub> values 1 and 0. The evaluation of chromatograms is by autoradiography after treatment with red ink, which contains radioactive sulphur <sup>35</sup>S.

### References

- 1) G. G. Brownlee and F. Sanger, *European J. Biochem.* **11** (1969) 395 – 399
- 2) B. E. Griffin, *FEBS Letters* **15** (1971) 165
- 3) F. Sanger et al., *J. Mol. Biol.* **13** (1965) 373 – 398.

### Characterisation of DNA adducts of a food mutagen by <sup>32</sup>P postlabelling

W. Pfau et al., *Carcinogenesis* **17** (1996) 2727-2732

2-Amino-3-methyl-9H-pyrido[2,3-b]indole (MeAaC) is a mutagenic and carcinogenic heterocyclic amine, which can be formed during frying or broiling of protein-containing food. In vitro as in vivo it forms covalent DNA adducts. After chemical reduction an important DNA adducts could be detected by <sup>32</sup>P postlabelling.

Layer: 20 x 20 cm, POLYGRAM® CEL 300 PEI, (Cat. No. 801053) cut to 10 x 10 cm

Sample application:

with a 10 x 7 cm wick of filter paper MN 440

Eluent:

1st dimension sodium phosphate buffer (1 M, pH 6.0)

2nd dimension (opposite to 1st dimension)

5.3 M lithium formate, 8.5 M urea, pH 3.5

3rd dimension

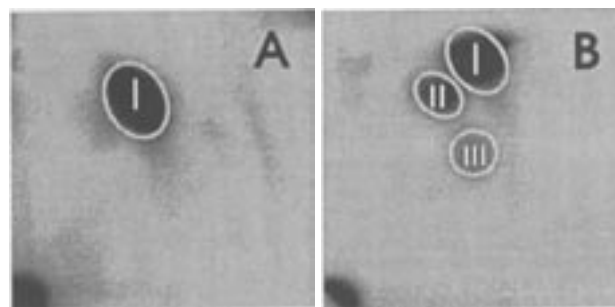
(perpendicular to 2nd dimension from left to right)

1.2 M lithium chloride, 0.5 M Tris/HCl, 8.5 M urea, pH 8.0

4th dimension 1.7 M sodium phosphate (pH 6.0)

wash and dry plate between individual developing steps

Detection: autoradiography at -80 °C with intensifying screens.



Autoradiography of the <sup>32</sup>P postlabelling / ion exchange TLC analysis of DNA from primary rat hepatocytes after treatment with MeAaC: A) butanol extraction, B) nuclease P1. I, II and III important adducts.

## Cellulose ion exchangers · ordering information

Designation	Thick-ness of layer	Plate size [cm]			Fluores-cent indicator
		5 x 20 50 / pack	20 x 20 25 / pack	40 x 20 25 / pack	
<b>Cellulose MN 300 DEAE</b> diethylaminoethyl-modified cellulose ion exchanger					
<b>POLYGRAM® polyester sheets</b>					
CEL 300 DEAE	0.10 mm	<b>801072</b>	<b>801073</b>	<b>801074</b>	–
<b>Cellulose MN 300 DEAE / MN 300 HR</b>					
mixed layers of cellulose ion exchanger MN 300 DEAE and high purity fibrous cellulose MN 300 HR					
<b>Glass plates</b>					
CEL DEAE/HR-MIX-20	0.20 mm			<b>810064</b>	–
<b>POLYGRAM® polyester sheets</b>					
CEL 300 DEAE/HR-2/15	0.10 mm			<b>801084</b>	–
<b>Cellulose MN 300 PEI</b> polyethyleneimine-impregnated cellulose ion exchanger					
<b>POLYGRAM® polyester sheets</b>					
CEL 300 PEI	0.10 mm	<b>801052</b>	<b>801053</b>	<b>801054</b>	–
CEL 300 PEI/UV <sub>254</sub>	0.10 mm	<b>801062</b>	<b>801063</b>	<b>801064</b>	UV <sub>254</sub>

# Ready-to-use layers for TLC



## Cellulose · TLC ready-to-use layers

### Acetylated cellulose

Acetylated cellulose (AC cellulose) is suited for reversed phase chromatography. It is prepared by esterification of cellulose with acetic acid. Up to three hydroxy groups per cellulose unit can be acetylated. The acetyl content can vary from a few percent to maximal 44.8%. This value corresponds to the cellulose triacetate. With increasing acetyl content the hydrophobic character of the AC powder also increases. With

a different degree of acetylation of the cellulose thus a continuous transition from a hydrophilic to a hydrophobic phase is obtained. For selection of the eluent it should be noted that the different esters, i.e. mono-, di- and triacetate are soluble in some organic solvents. We supply ready-to-use layers with 10, 20, 30 and 40% AC cellulose (absolute contents), designated AC-10, AC-20, AC-30 and AC-40.

### Ordering information

Designation	Thick-ness of layer	Acetyl content	Plate size [cm]		Fluorescent indi-cator
			5 x 20 50 / pack	20 x 20 25 / pack	
<b>Acetylated cellulose MN 300</b> fibrous cellulose, acetylated					
<b>Glass plates</b>					
CEL 300-10/AC-10%	0.10 mm	10 %		<b>808113</b>	–
CEL 300-10/AC-20%	0.10 mm	20 %		<b>808123</b>	–
CEL 300-10/AC-30%	0.10 mm	30 %		<b>808133</b>	–
CEL 300-10/AC-40%	0.10 mm	40 %		<b>808143</b>	–
CEL 300-10/AC-40% UV <sub>254</sub>	0.10 mm	40 %		<b>808144</b>	UV <sub>254</sub>
<b>POLYGRAM® polyester sheets</b>					
CEL 300 AC-10%	0.10 mm	10 %	<b>801032</b>	<b>801033</b>	–
CEL 300 AC-30%	0.10 mm	30 %	<b>801042</b>	<b>801043</b>	–

## Polyamide · TLC ready-to-use layers

Our ready-to-use layers with polyamide are coated with polyamide 6, i. e. perlon (= ε-aminopolycaprolactam). They can be supplied with or without an acid resistant UV indicator with a fluorescence at 254 nm. Due to the amide groups of the polymer matrix this phase can form hydrogen bonds with numerous classes of compounds and thus allows separations which are not possible with other adsorbents. Ready-to-use TLC sheets with polyamide are especially suited for natural compounds, phenols, carboxylic acids and aromatic nitro compounds. For a detailed discussion of the chromatographic properties of polyamide please refer to the chapter "Adsorbents for TLC" on page 296.

The polyamide phase has gained special importance for the analysis of amino acids. The figure on the right shows a two-dimensional thin layer chromatogram of dansylated amino acids on this layer.

### Separation of dansyl amino acids

Layer: POLYGRAM® Polyamide-6  
 Eluent: 1st dimension: 1.5% (v/v) aqueous formic acid  
 2nd dimension: toluol / glacial acetic acid (10:1, v/v)  
 Migration distance: 9 cm each in both dimensions  
 Detection: fluorescence

#### Peaks:

- 1.) DNS-leucine
- 2.) DNS-phenylalanine
- 3.) DNS-proline
- 4.) DNS-valine
- 5.) DNS-alanine
- 6.) DNS-glycine



### Ordering information

Designation	Thickness of layer	Plate size [cm]		Fluorescent indi-cator
		5 x 20 50 / pack	20 x 20 25 / pack	
<b>POLYAMIDE-6</b> = perlon = ε-aminopolycaprolactame				
<b>POLYGRAM® polyester sheets</b>				
POLYAMIDE-6	0.10 mm	<b>803012</b>	<b>803013</b>	–
POLYAMIDE-6 UV <sub>254</sub>	0.10 mm	<b>803022</b>	<b>803023</b>	UV <sub>254</sub>

# Ready-to-use layers for TLC

## Special ready-to-use layers for TLC / HPTLC

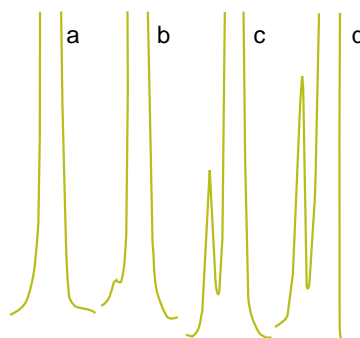
### CHIRALPLATE · the first TLC plate for control of optical purity

Gas chromatographic and high performance liquid chromatographic separations of enantiomers have been extensively studied. These methods require costly equipment and sometimes sample derivatisation is necessary. Hence, development of a chiral TLC plate – called CHIRALPLATE – was highly desirable to achieve simple, rapid highly sensitive and effective control of optical purity. CHIRALPLATE has been developed several years ago in co-operation with Degussa AG, Hanau, Germany.

#### Separation mechanism

CHIRALPLATE is a TLC glass plate coated with a reversed phase silica gel, and impregnated with a chiral selector (a proline derivative, DP 31 43 726 and EP 0 143 147) and copper(II) ions. It allows multiple simultaneous separations, a great advantage for routine applications and production control. The separation of optically active isomers is based on ligand exchange: the chiral selector on the plate and the enantiomers to be separated form diastereomeric mixed chelate complexes with the transition metal ion (copper in the case of CHIRALPLATE). Complexes for the different antipodes have different stabilities thus achieving chromatographic separation.

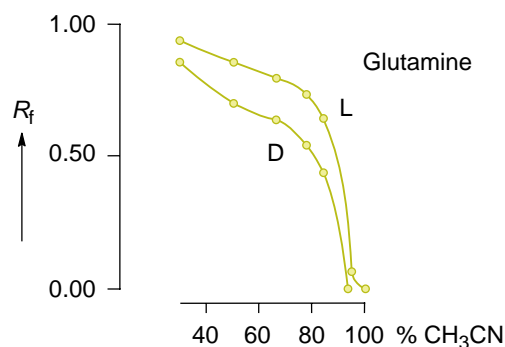
#### Enantiomer separation of amino acids <sup>2),6)</sup>



Quantitative determination (remission location curves) of TLC-separated enantiomers of *tert*-leucine: a) *L-tert*-leucine, b) *L-tert*-leucine + 0.1% *D-tert*-leucine, c) *L-tert*-leucine + 1 % *D-tert*-leucine, d) external reference sample  
Layer: CHIRALPLATE, eluent: methanol – water (10:80, v/v), detection: dip in 0.3% ninhydrin solution  
quantification with scanner, 520 nm

#### Chromatographic conditions for use of CHIRALPLATE

Our CHIRALPLATE is a ready-to-use plate. No circumstantial dippings in different solvents or solutions are necessary. For amino acid derivatives most of the separations achieved so far have been run with a mixture of methanol – water – acetonitrile in the ratio 50:50:200 v/v/v (eluent A, developing time about 30 minutes) or 50:50:30 v/v/v (eluent B, developing time about 60 minutes). By changing the amount of acetonitrile in the developing solvent you can influence efficiency and separation time. Lowering the acetonitrile concentration rapidly increases separation times. U. A. Th. Brinkman et al.<sup>1)</sup> investigated the influence of the composition of different binary mixtures of acetonitrile and water on the separation of different amino acid racemates (see fig. below)



Dependence of the  $R_f$  values of the two antipodes of D,L-glutamine from the acetonitrile content of the eluent <sup>1)</sup>

Though the above mentioned solvent mixtures are useful to separate many substances, in special cases other eluents may be required <sup>2)</sup>. Thus leucine can be separated in a mixture of methanol – water (10:80 v/v, developing time about 90 min); alanine and serine can be resolved with acetone – methanol – water (10:2:2 v/v/v, developing time ~ 50 min); N-carbamyl-tryptophan is separated with 1 mM cupric acetate solution containing 5% methanol (pH 5.8) <sup>3)</sup> and enantiomeric  $\alpha$ -hydroxycarboxylic acids could be resolved using dichloromethane – methanol (45:5 v/v, developing time about 20 min) <sup>2), 4)</sup>.

#### Ordering information

Designation	Thickness of layer	Plate size [cm]				Fluorescent indicator
		5 x 20	10 x 10	10 x 20	20 x 20	
<b>CHIRALPLATE</b> RP silica coated with $\text{Cu}^{2+}$ ions and chiral reagent, for enantiomer separation						
<b>Glass plates</b>						
CHIRALPLATE	0.25 mm			4 / pack <b>811056</b>		UV <sub>254</sub>
CHIRALPLATE	0.25 mm	50 / pack <b>811057</b>	25 / pack <b>811059</b>	25 / pack <b>811055</b>	25 / pack <b>811058</b>	UV <sub>254</sub>



## Special ready-to-use layers for TLC / HPTLC

For optimal separations and reproducible  $R_f$  values we recommend activation of plates prior to spotting (15 min at 100 °C) and use of chamber saturation for development. According to U. A. Th. Brinkman et al. <sup>1)</sup>, however, in some cases good separations can be achieved in 5 minutes without activation and without chamber saturation.

Nyiredy et al. <sup>5)</sup> described the application of CHIRALPLATE in forced-flow planar chromatography.

### Detection and quantitative determinations

Different detection methods are required for the different compounds separated: for proteinogenic and nonproteinogenic amino acids plates are dipped for 3 sec in a 0.3% ninhydrin solution in acetone (or sprayed with ninhydrin spray reagent), then dried for about 5 minutes at 110 °C. Red derivatives are formed on a white background. Vanadium pentoxide is especially useful for post-chromatographic derivatisation of  $\alpha$ -hydroxycarboxylic acids. As shown by Günther <sup>2), 6)</sup>, quantitative evaluation of antipodes separated on CHIRALPLATE is easily accomplished using a double beam scanner or a densitometer. In order to enhance specificity and sensitivity, post-chromatographic derivatisation with ninhydrin or vanadium pentoxide is recommended. This method allows determination of trace levels down to 0.1% of one enantiomer in an excess of the other.

### Application of CHIRALPLATE

Applications published so far include separations of

- amino acids
- N-methylamino acids
- N-formylamino acids
- $\alpha$ -alkylamino acids
- thiazolidine derivatives
- dipeptides
- lactones
- $\alpha$ -hydroxycarboxylic acids

and others <sup>7) - 14)</sup>. Feldberg and Reppucci describe the TLC separation of anomeric purine nucleosides on CHIRALPLATE <sup>15)</sup>. A review on the application of CHIRALPLATE has been given by Günther <sup>2)</sup>.

Procedures for the control of optical purity of L-dopa <sup>16)</sup> and D-penicillamine <sup>17)</sup> prove effectiveness of our CHIRALPLATE for pharmaceutical applications. In addition to the separation of enantiomers CHIRALPLATE shows increased selectivity for diastereomers as shown for D,L-alanyl-D-phenylalanine and L-alanyl-D,L-phenylalanine.

Separations of other groups of optically active compounds seem possible, if these compounds can be separated by ligand exchange, i.e. if they can form chelate complexes with copper(II) ions.

The following tabulated summaries show some examples of the compounds separated so far. For applications see our interactive collection of chromatography applications on the internet:

### TLC separation of proteinogenic and non-proteinogenic amino acids

To date 12 proteinogenic amino acids could be separated on CHIRALPLATE without derivatisation. Cysteine can be determined as thiazolidine-4-carboxylic acid after a simple derivatisation step. The table lists the proteinogenic and non-proteinogenic amino acids separated

Compound	$R_f$ value (configuration)		eluent
Alanine	0.69 (D)	0.73 (L)	D
Aspartic acid	0.50 (D)	0.55 (L)	A
Glutamic acid	0.54 (D)	0.59 (L)	A
Glutamine	0.41 (L)	0.55 (D)	A
Isoleucine	0.47 (D)	0.58 (L)	A
Leucine	0.53 (D)	0.63 (L)	C
Methionine	0.54 (D)	0.59 (L)	A
Valine	0.54 (D)	0.62 (L)	A
Phenylalanine	0.49 (D)	0.59 (L)	A
Serine	0.73 (D)	0.76 (L)	D
Tyrosine	0.58 (D)	0.66 (L)	A
Tryptophan	0.51 (D)	0.61 (L)	A
Proline	0.41 (D)	0.47 (L)	A
Cysteine as thiazolidine-4-carboxylic acid	0.59 (D)	0.69 (L)	A
<i>tert.</i> -Leucine	0.40 (D)	0.51 (L)	A
Norleucine	0.53 (D)	0.62 (L)	A
<i>allo</i> -Isoleucine	0.51 (D)	0.61 (L)	A
Norvaline	0.49 (D)	0.56 (L)	A
Homophenylalanine <sup>14)</sup>	0.49 (D)	0.58 (L)	A
<i>allo</i> -4-Hydroxyproline	0.41 (L)	0.59 (D)	A
2-Phenylglycine	0.57 (D)	0.67 (L)	A
2-Cyclopentylglycine	0.43	0.50	A
3-Cyclopentylalanine <sup>14)</sup>	0.46	0.56	A
2-(1-Methylcyclopropyl)-glycine <sup>14)</sup>	0.49	0.57	A
Ethionine	0.52 (D)	0.59 (L)	A
1-Naphthylalanine	0.49 (D)	0.56 (L)	A
2-Naphthylalanine	0.44 (D)	0.59 (L)	A
O-Benzylserine	0.54 (D)	0.65 (L)	A
O-Benzyltyrosine	0.48 (D)	0.64 (L)	A
4-Methyltryptophan	0.50	0.58	A
5-Methyltryptophan <sup>14)</sup>	0.52	0.63	A
6-Methyltryptophan <sup>14)</sup>	0.52	0.64	A
7-Methyltryptophan <sup>14)</sup>	0.51	0.64	A
4-Methoxyphenylalanine	0.52	0.64	A
5-Methoxytryptophan	0.55	0.66	A
Methionine sulphone	0.62 (D)	0.66 (L)	A
Ethionine sulphone	0.55	0.59	A
Selenomethionine	0.53 (D)	0.61 (L)	A
Dopa	0.47 (L)	0.58 (D)	B
(= 3,4-dihydroxyphenylalanine)			

migration distance 13 cm, chamber saturation  
 eluent A: methanol/water/acetonitrile (50:50:200, v/v/v)  
 eluent B: methanol/water/acetonitrile (50:50:30, v/v/v)  
 eluent C: methanol/water (10:80, v/v)  
 eluent D: acetone/methanol/water (10:2:2, v/v/v)

# Ready-to-use layers for TLC

## Special ready-to-use layers for TLC / HPTLC

### TLC enantiomer separation of dipeptides

For the enantiomer separation of dipeptides it is remarkable, that the enantiomer with C-terminal L-configuration always has a lower  $R_f$  value than the enantiomer with C-terminal D-configuration. The method can resolve diastereomeric dipeptides as well <sup>9)</sup>

Compound	$R_f$ value (configuration)		eluent
D,L-Alanyl-D-phenylalanine	0.25	0.32	*A
L-Alanyl-D,L-phenylalanine	0.27	0.34	*A
Gly-D,L-Phe	0.57 (L)	0.63 (D)	B
Gly-D,L-Leu	0.53 (L)	0.60 (D)	B
Gly-D,L-Ileu	0.54 (L)	0.61 (D)	B
Gly-D,L-Val	0.58 (L)	0.62 (D)	B
Gly-D,L-Trp	0.48 (L)	0.55 (D)	B
D-Leu-L-Leu	0.48		B
L-Leu-D-Leu	0.57		B
D-Leu-L-Leu	0.19		A
L-Leu-D-Leu	0.26		A
D-Ala-L-Phe	0.59		B
L-Ala-D-Phe	0.65		B
D-Ala-L-Phe	0.21		A
L-Ala-D-Phe	0.26		A
D-Met-L-Met	0.64		B
L-Met-D-Met	0.71		B
D-Met-L-Met	0.29		A
L-Met-D-Met	0.33		A

\* = diastereomer separation

migration distance 13 cm, chamber saturation  
eluent A: methanol/water/acetonitrile (50:50:200, v/v/v)  
eluent B: methanol/water/acetonitrile (50:50:30, v/v/v)

### TLC separation of enantiomeric $\alpha$ -methylamino acids

Compound	$R_f$ value (configuration)		eluent
$\alpha$ -Methyl- $\alpha$ -aminobutyric acid	0.50	0.60	A
$\alpha$ -Methyl- $\alpha$ -aminocaproic acid	0.51	0.55	A
$\alpha$ -Methylaspartic acid	0.52 (D)	0.56 (L)	A
$\alpha$ -Methyl-dopa <sup>1)</sup>	0.46 (L)	0.66 (D)	B
$\alpha$ -Methylglutamic acid	0.58 (L)	0.62 (D)	A
$\alpha$ -Methylleucine	0.48	0.59	A
$\alpha$ -Methylmethionine	0.56 (D)	0.64 (L)	A
$\alpha$ -Methylphenylalanine	0.53 (L)	0.66 (D)	A
$\alpha$ -Methylphenylglycine	0.58	0.69	A
$\alpha$ -Methylserine	0.56 (L)	0.67 (D)	B
$\alpha$ -Methyltryptophan	0.54	0.65	A
$\alpha$ -Methyltyrosine	0.63 (D)	0.70 (L)	A
$\alpha$ -Methylvaline	0.51	0.56	A

migration distance 13 cm, chamber saturation  
eluent A: methanol/water/acetonitrile (50:50:200, v/v/v)  
eluent B: methanol/water/acetonitrile (50:50:30, v/v/v)

### TLC separation of enantiomeric N-alkyl and N-formyl amino acids

Compound	$R_f$ value (configuration)		eluent
N-Methylleucine	0.49 (L)	0.57 (D)	A
N-Methylphenylalanine	0.50 (D)	0.61 (L)	A
N,N-Dimethylphenylalanine	0.55 (D)	0.61 (L)	B
N-Methyl-m-tyrosine	0.36	0.52	B
N-Methylvaline	0.65 (L)	0.70 (D)	B
N-Formyl- <i>tert.</i> -leucine	0.48 (+)	0.61 (-)	A

migration distance 13 cm, chamber saturation  
eluent A: methanol/water/acetonitrile (50:50:200, v/v/v)  
eluent B: methanol/water/acetonitrile (50:50:30, v/v/v)

### TLC separation of halogenated amino acids

Compound	$R_f$ value (configuration)		eluent
3-Chloroalanine	0.57	0.64	A
4-Bromophenylalanine	0.44	0.58	A
4-Chlorophenylalanine	0.46	0.59	A
2-Fluorophenylalanine	0.55	0.61	A
4-Iodophenylalanine	0.45 (D)	0.61 (L)	A
5-Bromotryptophan	0.46	0.58	A
3-Fluorotyrosine	0.64	0.71	A
Thyroxine	0.38 (D)	0.49 (L)	A

migration distance 13 cm, chamber saturation  
eluent A: methanol/water/acetonitrile (50:50:200, v/v/v)

### TLC enantiomer separation of different classes of compounds

Compound	$R_f$ value (configuration)		eluent
N-Carbamyltryptophan <sup>3)</sup>	0.44 (L)	0.55 (D)	C
N-Glycylleucine	0.48 (L)	0.56 (D)	B
N-Glycylphenylalanine <sup>8)</sup>	0.51 (L)	0.57 (D)	B
2-Aminobutyric acid	0.48	0.52	A
3-Amino-3,5,5-trimethylbutyrolactone · HCl	0.50	0.59	A
4-Aminophenylalanine	0.33	0.47	A
3,4-Dihydroxyphenylalanine (= Dopa)	0.47 (L)	0.58 (D)	B
4-Nitrophenylalanine	0.52	0.61	A
Thiazolidine-4-carboxylic acid	0.59 (D)	0.69 (L)	A
5,5-Dimethylthiazolidine-carboxylic acid hydrochloride (from penicillamine)	0.48 (D)	0.62 (L)	A
S-(2-Chlorobenzyl)-cysteine	0.45	0.58	A
S-(3-Thiabutyl)-cysteine	0.53	0.64	A
S-(2-Thiapropryl)-cysteine	0.53	0.64	A
Pipecolic acid	0.51	0.58	D

eluent A: methanol/water/acetonitrile (50:50:200, v/v/v)  
eluent B: methanol/water/acetonitrile (50:50:30, v/v/v)  
eluent C: 1 mM Cu(II) acetate, 5% methanol pH 5.8, 16 °C  
eluent D: acetone/methanol/water (10:2:2, v/v/v)



## Special ready-to-use layers for TLC / HPTLC

### TLC enantiomer separation of $\alpha$ -hydroxycarboxylic acids

Jork and Kany <sup>4)</sup> for the first time succeeded in separating enantiomeric  $\alpha$ -hydroxycarboxylic acids. Mandelic acid is gaining increasing importance as a multifunctional chiral building block in preparative chemistry. For this reason an economical method for control of optical purity of this class of compounds was highly desirable. Günther <sup>2)</sup> describes the separation of  $\alpha$ -hydroxycarboxylic acids on CHIRALPLATE using the lipophilic solvent mixture dichloromethane/methanol (45:5, v/v). Vanadium pentoxide is especially useful for visualisation of these compounds by postchromatographic derivatisation.

Compound	$R_f$ value (configuration)	
Mandelic acid	0.46	0.53 (L)
3-Hydroxymandelic acid	0.34	0.39
4-Hydroxymandelic acid	0.32	0.36
3,4-Dihydroxymandelic acid	0.32	0.38
Vanillylmandelic acid	0.49	0.55
Hydroxyisoleucine	0.56	0.63 (L)
Hydroxyleucine (sodium salt)	0.56	0.60 (L)
Hydroxymethionine (sodium salt)	0.52	0.58 (L)
Hydroxyphenylalanine	0.56	0.62 (L)
Hydroxyvaline	0.52	0.60 (L)

migration distance 13 cm  
eluent: dichloromethane / methanol (45:5, v/v)

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For ordering information of CHIRALPLATE please see page 282.

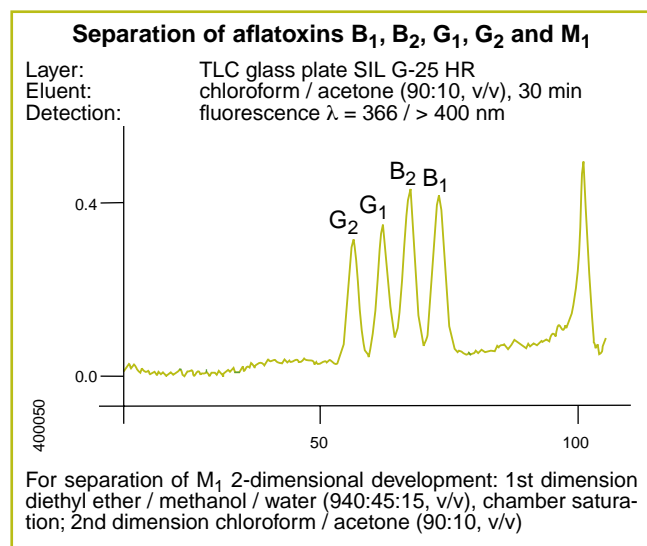
# Ready-to-use layers for TLC

## Special ready-to-use layers for TLC / HPTLC

### Ready-to-use TLC plates SIL G-25 HR for separation of aflatoxins

For analytical detection of aflatoxins – the extremely toxic metabolic products of mold – thin layer chromatography is used as a routine method. Detection is possible under UV light. A test of numerous adsorbents and ready-to-use layers showed, that our silica G-HR and the corresponding glass plates SIL G-25 HR give excellent results for the separation of aflatoxins. TLC separations with layers of silica G-HR are mentioned in numerous publications.

The layer of the glass plate SIL G-25 HR contains gypsum as binder and a very small quantity of an organic highly polymeric compound. The layer is considerably softer than for the standard plate and therefore especially suited for separations, where substance spots are to be scratched from the plate or for cases, where fast-absorbing layers are preferred.



### TLC ready-to-use plates SIL G-25 TENSIDE for the separation of detergents, alkanesulphonates, polyglycols etc. and for assessment of fetal lung maturity

The TLC separation of surfactants on layers of silica G – impregnated with ammonium sulphate – is recommended in a number of publications. For either personnel or technical reasons many laboratories no longer have the possibility to prepare TLC plates with special impregnation. This is why we supply a TLC glass plate coated with ammonium sulphate impregnated silica G.

For the assessment of fetal lung maturity it is essential to determine the ratio lecithin/sphingomyelin and detect the presence of phosphatidylglycerol in amniotic fluid. Both determinations are possible after one TLC separation on SIL G-25 TENSIDE.

### Investigation of amniotic fluid extracts for assessment of the neonatal respiratory syndrome

M.J. Whittle, A.I. Wilson, C.R. Whitfield, R.D. Paton, R.W. Logan, Br. J. Obstet. Gynaecol. **89** (1982) 727-732

Exact prediction of the neonatal respiratory syndrome requires determination of the lecithin/sphingomyelin (L/S) ratio as well as detection of phosphatidylglycerol (PG) in amniotic fluid. Cases L/S  $\geq 2$  or  $< 2$  and PG present or absent are of significance. Both determinations are possible in one TLC separation on SIL G-25 TENSIDE.

Layer: TLC glass plate SIL G-25 TENSIDE  
 Laufmittel: 2-dimensional development  
 1st dimension chloroform / methanol / water / glacial acetic acid (65:25:4:8, v/v)  
 2nd dimension tetrahydrofuran / formaldehyde / methanol / 2 N ammonium hydroxide (40:28,5:7,8:4,2, v/v)

Migration distance: 2 x 10 cm in 30 minutes each, ascending  
 Detection: charring (10 min at 250 °C), then planimetric determination of the L/S ratio, check whether PG present or absent

400730

### TLC ready-to-use layers with kieselguhr

TLC ready-to-use layers with kieselguhr coating are not very significant. Kieselguhr is completely inactive and for this reason is mostly used for special separations after suitable

impregnation, e.g. impregnation with EDTA for separation of tetracycline broad-spectrum antibiotics (see DAB 8 and EAB Vol. II, p. 360).

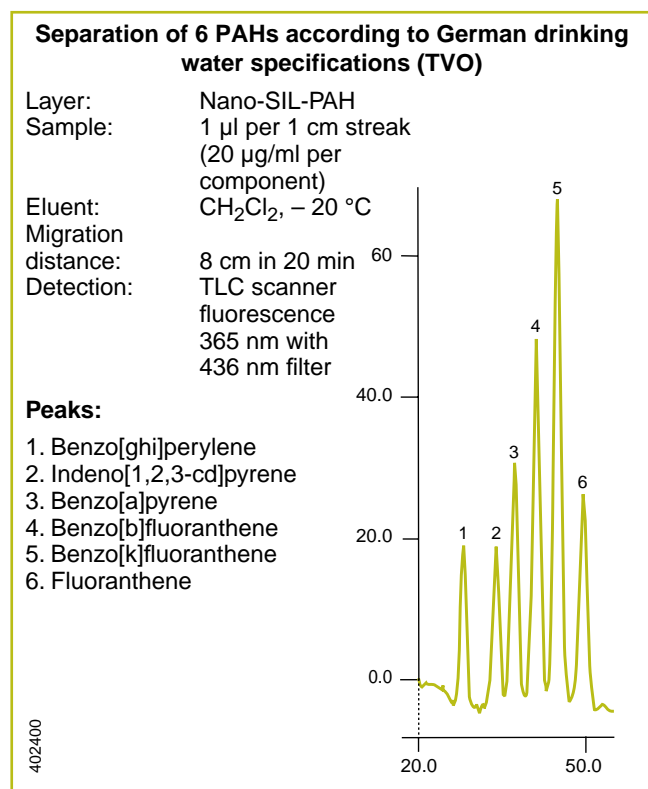
### Ordering information - Glass plates

Designation	Thickness of layer	Plate size [cm] 20 x 20 25 Stück	Fluorescent indicator
<b>SIL G-25 HR</b> high purity silica 60 with gypsum, recommended for aflatoxin separations			
SIL G-25 HR	0.25 mm	<b>809033</b>	–
SIL G-25 HR/UV <sub>254</sub>	0.25 mm	<b>809043</b>	UV <sub>254</sub>
<b>SIL G-25 Tenside</b> silica G with ammonium sulphate for separation of surfactants			
SIL G-25 Tenside	0.25 mm	<b>810063</b>	–
<b>GUR N</b> TLC ready-to-use layers with kieselguhr			
GUR N-25	0.25 mm	<b>810074</b>	–
GUR N-25 UV <sub>254</sub>	0.25 mm	<b>810073</b>	UV <sub>254</sub>



## Special ready-to-use layers for TLC / HPTLC

### Nano-SIL-PAH HPTLC ready-to-use layer for separation of polycyclic aromatic hydrocarbons



For TLC separation of polycyclic aromatic hydrocarbons (PAH) special TLC layers have been described<sup>1)–3)</sup>, which have been impregnated or dipped with electron acceptors of different strengths. The PAHs act as electron donors and with these electron acceptors form charge-transfer complexes, which can be separated chromatographically. Funk et al.<sup>4),5)</sup> developed a method, in which TLC ready-to-use plates are dipped with the electron acceptor caffeine and which separates the six PAHs according to the German drinking water specifications (TVO). This method of impregnation by dipping is also recommended in the draft for the German standard DIN 38407, part 7 “Determination of six polycyclic aromatic hydrocarbons in drinking water and mineral water via HPTLC”. In order to save our customers the circumstantial dipping of plates, we have developed the HPTLC ready-to-use plate **Nano-SIL-PAH**. This plate can – without dipping – be directly used for the method recommended in the draft of the German standard DIN 38407, part 7, because the layer already contains the required electron acceptor. As adsorbent the plate is coated with our well-known nano-silica with 60 Å pore size and 2 – 10 µm particle size. Due to the outstanding separation performance of Nano-SIL-PAH the six PAHs according to TVO can be very well quantitated.

#### References

- 1) A. Berg, J. Lam, J. Chromatogr. **16** (1964) 157 – 166
- 2) M. A. Sliifkin, S. H. Liu, J. Chromatogr. **269** (1983) 103 – 107
- 3) M. A. Sliifkin, H. Singh, J. Chromatogr. **303** (1984) 190 – 192
- 4) W. Funk et al., J. Planar Chromatogr. **2** (1992) 28 – 32
- 5) W. Funk et al., J. Planar Chromatogr. **2** (1992) 317 – 320

#### Ordering information

Designation	Thickness of layer	Plate size [cm]		Fluorescent indicator
		10 x 10	10 x 20	
		Pack of 25	50	
<b>Nano-SIL PAH</b> nano silica with special impregnation for PAH analysis				
<b>Glass plates</b>				
Nano-SIL-PAH	0.20 mm	<b>811050</b>	<b>811051</b>	–

#### TLC ready-to-use layers with ion exchange resins

POLYGRAM® IONEX-25 precoated sheets are polyester sheets coated with a mixture of silica and a cation or anion exchange resin, respectively. Additionally these layers contain an inert organic binder. They are suited for separation of amino acids, e.g. in protein and peptide hydrolysates, in

seeds and fodder, in biological fluids, for racemate separation in peptide syntheses, for the separation of nucleic acid hydrolysates, aminosugars, aminocarboxylic acids, antibiotics, inorganic phosphates, cations and other compounds with ionic groups.

#### Ordering information

Designation	Thickness of layer	Plate size [cm]		Fluorescent indicator
		20 x 20		
		Pack of 25		
<b>IONEX</b> TLC ready-to-use layers with mixed layers of ion exchange resins / silica				
<b>POLYGRAM® polyester sheets</b>				
IONEX -25 SA- Na	strongly acidic cation exchanger	0.20 mm	<b>806013</b>	–
IONEX-25 SB-AC	strongly basic anion exchanger	0.20 mm	<b>806023</b>	–
IONEX-25 SB-AC/UV <sub>254</sub>	strongly basic anion exchanger	0.20 mm	<b>806033</b>	UV <sub>254</sub>

# Ready-to-use layers for TLC

## Special ready-to-use layers for TLC / HPTLC

### Mixed layers - TLC ready-to-use plates

#### TLC ready-to-use plates ALOX/CEL-AC-Mix-25 for separation of polycyclic aromatic hydrocarbons (PAHs)

Polycyclic aromatic hydrocarbons (PAHs) are among the most frequent carcinogens in the environment. For this reason environmental agencies in most countries define maximum allowable concentrations e.g. in drinking water, which have to be frequently controlled. A successful analytical procedure is based on fluorescence measurement after TLC separation on mixed layers of aluminium oxide G and acetylated cellulose. We supply corresponding glass plates with mixed layers under the designation ALOX/CEL-AC-Mix-25.

H. Hellmann [Z. Anal. Chem. **295** (1979) 24] notes that the fluorescence intensity of the individual compounds can vary considerably during separation as well as later on, which is important for the evaluation of a chromatogram.

For separation of polycyclic aromatic hydrocarbons you can also use layers of acetylated cellulose without addition of aluminium oxide, our wettable TLC plate RP-18 W/UV<sub>254</sub> or the specially impregnated plate Nano-SIL-PAH.

#### TLC ready-to-use plates GURSIL-Mix-25 UV<sub>254</sub>

In thin layer chromatography, kieselguhr is not only used as pure adsorbent, but also as mixed layer with silica for the separation of carbohydrates, antioxidants, steroids and photographic developer substances. MACHEREY-NAGEL offers a TLC plate for this purpose under the name GURSIL-Mix-25 UV<sub>254</sub>. These mixed layers show a reduced adsorption capacity compared to plain silica layers and allow rapid separations. Carbohydrates such as glucose, sorbitol and mannitol can be separated on this layer with isopropanol/ethyl acetate/water (83:11:6, v/v) as eluent. The eluent carbon tetrachloride/glacial acetic acid (75:25, v/v) is suited for the separation of photographic developer substances such as pyrocatechol, hydroquinone, pyrogallol, 1-phenyl-3-pyrazolidone and *p*-phenylene diamine.

#### Determination of polycyclic aromatic hydrocarbons (PAHs) according to German drinking water specifications

Investigation of potential interference with other PAHs

H. Kunte, Fresenius Z. Anal. Chemie **301** (1980) 287-289

Layer: TLC glass plate ALOX/CEL-AC-Mix-25

Eluent: 1st dimension: *n*-hexane / benzene\* (9 : 1)

2nd dimension: methanol / diethylether / water (4 : 4 : 1)

Detection: fluorescence

\* Instead of benzene we recommend the less toxic toluene.

401040

#### TLC ready-to-use plates SILCEL-Mix-25 UV<sub>254</sub> for separation of preservatives

Layers of polyamide 11 UV<sub>254</sub> were successfully used for many years especially for the separation of food preservatives [see J. Clement et al., Z. Anal. Chem. **248** (1969) 182]. Unfortunately, polyamide 11 is no longer produced. For this reason we have developed – based on a publication of J. A. W. Gosselé [J. Chromatogr. **63** (1971) 433] a TLC glass plate with a mixed layer of cellulose and silica, which is extremely well suited for the separation of preservatives (benzoic acid, sorbic acid, salicylic acid, *p*-hydroxybenzoic acid and lower *p*-hydroxybenzoates).

Our TLC glass plate SILCEL-Mix-25 UV<sub>254</sub> with the mixed layer of silica and cellulose is also very well suited for separation of numerous other antimicrobial compounds.

#### Separation of preservatives

J.A.W. Gosselé, J. Chromatogr. **63** (1971) 433

For separation of preservatives mixed layers of silica and cellulose are especially suited. This paper also describes several visualisation reagents for the substances investigated.

Layer: TLC glass plate SILCEL-Mix-25 UV<sub>254</sub>  
 Eluent: petroleum ether(40/60) / carbon tetrachloride / chloroform / formic acid / glacial acetic acid (50:40:20:8:2, v/v, upper phase)

Migration distance: 16 cm ascending, 2-fold development, chamber saturation

Detection: UV 254 nm

401420

### Ordering information

Designation	Thickness of layer	Plate size [cm]			Fluorescent indicator
		5 x 20 100 / pack	10 x 20 50 / pack	20 x 20 25 / pack	
<b>Mixed layers</b>					
<b>Glass plates</b>					
<b>Aluminium oxide G / acetylated cellulose</b>					
ALOX/CEL-AC-Mix-25	0.25 mm		<b>810054</b>	<b>810053</b>	–
<b>Cellulose / silica</b>					
SILCEL-Mix-25 UV <sub>254</sub>	0.25 mm		<b>810042</b>	<b>810043</b>	UV <sub>254</sub>
<b>Kieselguhr / silica</b>					
GURSIL-Mix-25 UV <sub>254</sub>	0.25 mm	<b>810077</b>	<b>810076</b>	<b>810075</b>	UV <sub>254</sub>



## Chromatography papers

Paper chromatography (PC) is the oldest chromatographic technique. As with the other chromatographic procedures separation is achieved by partition of the mixture to be separated between two immiscible phases. The stationary phase, for PC the chromatography paper, is intensely penetrated by the mobile phase through capillary action.

In normal paper chromatography the cellulose of the paper, which is loaded with water, forms the stationary phase, as mobile phase organic solvents or solvent mixtures with limited miscibility with water are used.

Contrary, for PC with reversed phases (RP chromatography) the paper is impregnated with hydrophobic solvents (e.g. formamide, dimethylformamide, paraffin oil, undecane, silicone oil), as mobile phase (eluent) hydrophilic solvents such as alcohols are used.

Strictly speaking the chromatography paper is only the support for the liquid stationary phase, however, the properties of the paper have a considerable influence on the quality of a separation.

For a paper chromatographic analysis about 1 – 2 µl of the 1 – 2% solution to be investigated is applied about 1.5 to 2 cm from the edge of a strip of chromatography paper with the aid of a micropipette and allowed to dry. Then the strip of paper is placed into a developing chamber (e.g. glass trough) charged with a suitable solvent mixture, such that the eluent can ascend in the paper by capillary action, but the substance spots are above the liquid level. The eluent will pass the starting points and transport the substances through the stationary phase, in this case the paper. Due to the different retention of substances on the paper surface separation of the mixture will be achieved.

When the eluent has almost reached the other end of the paper, remove the strip from the developing chamber, mark the solvent front with a lead pencil and dry the paper. As in thin layer chromatography for PC substances have to be visualised after separation.

Ascending, descending and circular techniques are also possible in paper chromatography.

Thus high quality filter paper is the medium for the separation process in PC. Papers used for PC are generally called chromatography papers, however, they are basically special

high-quality filter papers. As raw material for chromatography papers mostly linters are used, i.e. short cotton fibres, which are no longer suited for textile application, but they are especially suited for the manufacture of filter papers. As compared to pulp linters possess an intrinsic high purity and a very high content of  $\alpha$ -cellulose. Last but not least linters are advantageous, because they have relatively long fibres and thus allow a defined grinding. This is an important prerequisite for the manufacture of homogeneous and flawless papers.

Chromatography papers and cartons have to be treated carefully. For example they should never be touched with fingers, because in addition to water the human skin transpires salts, fats, amino acids and other substances, which can interfere with the separation.

Chromatography papers should not be bent sharply, because this will decrease the capillary action. For this reason sheets should preferably be stored flat, in any case never rolled to narrow.

Due to the manufacturing process in the paper machine the fibres will assume a preferred direction. For this reason every chromatography paper will show slightly different absorptive properties in different directions: in the direction of the fibres the absorption is generally higher than vertical to it. This is why for chromatography one should always work in the direction of higher absorption. For our sheets 58 x 60 cm this is the longer edge.

If for smaller cuts of a sheet the preferred direction can no longer be determined from the ratio of the edges, simply apply a drop of water to the paper: the spot will assume an elliptical shape, the longer axis showing the preferred direction.

The most important parameters for chromatography papers are weight, thickness and migration distance. For chromatography papers the migration distance is usually given in mm / 30 minutes, though normally in the paper industry the migration distance according to Klemm is used, i.e. the height of a strip of 15 mm width, which is wetted in 10 minutes when dipped in dist. water of 20 °C.

## Ordering information

Code	Weight [g/m <sup>2</sup> ]	Thickness [mm]	Description	Flow rate	Size [cm]	Pack of	Cat. No.
MN 214	140	0.28	smooth	90 – 100 mm/30 min	58 x 60	100 sheets	<b>817001</b>
MN 218	180	0.36	smooth	90 – 100 mm/30 min	58 x 60	100 sheets	<b>817002</b>
MN 260	90	0.20	smooth	130 – 150 mm/30 min	58 x 60	100 sheets	<b>817003</b>
MN 261	90	0.18	smooth	90 – 100 mm/30 min	58 x 60	100 sheets	<b>817004</b>
MN 827	270	0.70	soft carton	130 – 140 mm/10 min	58 x 60	100 sheets	<b>817005</b>
MN 866	650	1.70	soft carton	150 – 160 mm/10 min	38 x 38	100 sheets	<b>817006</b>
MN 866	650	1.70	soft carton	150 – 160 mm/10 min	80 x 80	100 sheets	<b>817007</b>
MN 214 ff	140	0.28	MN 214 defatted *	90 – 100 mm/30 min	56 x 58	100 sheets	<b>817008</b>

\*) This paper is extracted with organic solvents

# TLC micro-sets

## TLC introductory kits for science education

### TLC micro-sets for thin layer chromatography – educational tools with high didactic value

For science education, we offer two types of kits:

- **The beginner's set**  
features separations with simple developing solvents; samples are coloured thus eliminating the need for visualisation. All equipment needed is contained in the set.
- **The advanced sets**  
require some experience and skill from the user. For these sets, some of the samples have to be pretreated before separation, and for identification of substances spray reagents have to be used. Prerequisite for all advanced experiments is a materials set, which can also be used as basic equipment for individual thin layer chromatographic experiments. The additional equipment required for the advanced experiments is usually present in any laboratory (e.g. air drier, fume hood, solvents, test tubes, drying ovens etc.).

#### TLC wine set

*Chromatographic rapid test for evaluating the conversion of malic acid to lactic acid in wine (2nd fermentation)*

The ratio of malic acid to lactic acid is an important criterion for evaluating the optimum time for bottling of a wine. As a time-saving, economic alternative, which can be performed in the field, MACHEREY-NAGEL have developed the **wine set**.

To promote introduction of thin layer chromatography into science education at schools, universities and training centers, we have designed TLC micro-sets which demonstrate both the speed of the method and the high level of performance of our POLYGRAM® precoated sheets. For this purpose as well as for rapid product control during manufacturing processes micro precoated sheets (size 4 x 8 cm) are particularly suited. Furthermore with these kits we wish to encourage further research and help find solutions to individual problems. TLC micro-sets are kits containing materials and chemicals for easy separations with thin layer chromatography. They are especially recommended as introduction to TLC for students. Each kit contains detailed explanations and instructions for the respective separations.

All types of TLC micro-sets make use of micro precoated sheets size 4 x 8 cm, and only the ascending chromatographic technique is used.

#### TLC wine set

This set is based on POLYGRAM® CEL 300 ready-to-use sheets (4 x 8 cm) and allows separation of lactic, malic and tartaric acid within 10 min **without** need for post-chromatographic treatment of the cellulose layer. Already during separation in the developing chamber the separation of the acids can be watched. Another advantage is that this chromatographic separation does not require any additional instrumentation such as air drier or drying cabinet, thus the test can be performed in the field.





## TLC introductory kits for science education

Basically, for TLC micro-sets the same steps apply as described in the chapter "Principle steps of a thin layer chromatographic separation". After application of the sample with a glass capillary and evaporation of the solvent the micro pre-coated sheet is developed. TLC micro-sets use a simple glass bottle with screw cap as developing tank. Place the micro pre-coated sheet into the glass bottle, which is charged with about 10 ml of the developing solvent. Screw the cap on to the bottle while you hold the glass firmly on the table, so that the surface of the developing solvent is not disturbed. Please take care, that the solvent does not unevenly touch or splash over the applied spots. The solvent ascends through the layer by capillary action and causes the substances to be separated. When the solvent front has reached the desired height (about 6 to 7 cm) stop the separation by removing the sheet from the development tank. Immediately mark the solvent front with a soft lead pencil.

After chromatographic separation all colourless substances have to be made visible. For our beginner's set we have chosen dyes, i.e. coloured substances, which can be recognised without further treatment. The substances separated with the advanced sets are colourless as is usually the case in practice. For visualisation you will use spray reagents as detailed in the instructions for the sets.

### TLC micro-set A for beginners

This kit contains all chemicals and accessories for the following separations:

- separation of the fat-soluble (lipophilic) dye mixture 1: butter yellow, indophenol, sudan blue II, sudan red G
- separation of a mixture of anthraquinone dyes (test dye mixture 2): blue 1, blue 3, green, green blue, red, violet 1, violet 3
- separation of a mixture of food dyes (test dye mixture 3): brilliant black BN, fast red E, erythrosins, yellow orange S, naphthol red S, ponceau 4 R, tartrazine
- separation of dyes from felt tip pens

### Contents of TLC micro-set A for beginners

1	manual
3	developing chambers
50	glass capillaries 1 µl
1	spotting guide
1	measuring cylinder 10 ml
50	polyester sheets 4 x 8 cm each of POLYGRAM® SIL G/UV <sub>254</sub> , ALOX N/UV <sub>254</sub> and CEL 300
8 ml	each of test dye mixture 1 (4 lipophilic dyes), test dyes sudan res G, and sudan blue II
8 ml	each of test dye mixture 2 (7 anthraquinone dyes), test dyes blue 1 and violet 2
8 ml	each of test dye mixture 3 (7 food dyes), test dyes yellow orange S, and brilliant black BN
100 ml	each of toluene, toluene/cyclohexane (2:1, v/v) chloroform/acetone (1:1, v/v) 2.5% sodium citrate solution 25% ammonia/2-propanol (5:3, v/v)
2	felt tip pens

### Ordering information

Designation	Pack of	Cat. No.
<b>TLC micro-set A for beginners</b>	1 kit	<b>814000</b>
<b>Replacement parts for TLC micro-set A</b>		
<b>Test dye mixture 1</b> , solution of 4 lipophilic dyes in toluene (components see above)	8 ml	<b>814001</b>
<b>Test dye mixture 2</b> , solution of 7 anthraquinone dyes in chloroform (components see above)	8 ml	<b>814002</b>
<b>Test dye mixture 3</b> , aqueous solution of 7 food dyes (components see above)	8 ml	<b>814003</b>
Collection of 4 <b>individual components of test dye mixture 1</b>	4 x 8 ml	<b>814011</b>
Collection of 7 <b>individual components of test dye mixture 2</b>	7 x 8 ml	<b>814012</b>
Collection of 7 <b>individual components of test dye mixture 3</b>	7 x 8 ml	<b>814013</b>
<b>Sodium citrate</b> , 2.5 g in 100 ml bottles to fill up with distilled water	2.5 g	<b>814029</b>
For spray reagents see page 300.		



# TLC micro-sets

## TLC introductory kits for science education

### TLC micro-set M

This kit is prerequisite for the separations with kits F 1 to F 3. In addition, it serves as basic equipment for the individual study of further thin layer chromatographic experiments.

#### Contents of TLC micro-set M (materials kit)

2 x 50 glass capillaries 1  $\mu$ l,  
2 spotting guides  
1 rubber cap for capillaries,  
1 measuring cylinder 10 ml,  
1 beaker 25 ml,  
2 developing chambers  
1 glass laboratory sprayer with rubber bulb  
1 plastic syringe 1 ml  
20 sheets filter paper MN 713 (15 x 21 cm)  
50 polyester sheets 4 x 8 cm each of POLYGRAM® SIL G/UV<sub>254</sub>, ALOX N/UV<sub>254</sub> and CEL 300

### TLC micro-set F 1

This kit contains all chemicals required

- for the separation of amino acids (test mixture, consisting of alanine, arginine, tryptophan and valine)
- for the separation of amino acids in urine
- for the separation of the heavy metal cations cobalt(II), copper(II), manganese(II), and nickel(II)

#### Contents of TLC micro-set F1

1 manual  
50 glass capillaries 1  $\mu$ l  
50 polyester sheets 8 x 4 cm each of POLYGRAM® SIL G/UV<sub>254</sub> and CEL 300  
100 ml each of *n*-butanol, ninhydrin spray reagent (0.2% in ethanol), acetone, 25% ammonia, rubeanic acid spray reagent  
50 ml each of 50% acetic acid, 18% hydrochloric acid  
8 ml each of the amino acid test mixture (see right), tryptophan and arginine reference solutions  
8 ml each of the heavy metal cation test mixture (see above), Co<sup>2+</sup>, Mn<sup>2+</sup>, and Ni<sup>2+</sup> reference solution

### TLC micro-set F 2

This kit contains all chemicals required

- for the analysis of edible fats
- as well as for analysis of fats and cholesterol in blood

#### Contents of TLC micro-set F2

1 manual  
50 glass capillaries 1  $\mu$ l  
50 polyester sheets 4 x 8 cm POLYGRAM® SIL G/UV<sub>254</sub>  
5 blood lancets, 5 disposable pipettes 25  $\mu$ l, 5 alcoholic pads, 5 sample vials 2 ml with lid  
3 sample vials 30 ml (for butter, margarine and edible oil)  
100 ml each of chloroform, methylene chloride, toluene and molybdato-phosphoric acid spray reagent  
50 ml acetone with calibrated pipette  
8 ml cholesterol reference solution

### TLC micro-set F 3

This kit contains all chemicals required

- for the separation of analgetics (pain relievers)
- and for drug analysis as shown for cinchona bark.

#### Contents of TLC micro-set F3

1 manual, 50 glass capillaries 1  $\mu$ l  
50 polyester sheets 4 x 8 cm POLYGRAM® SIL G/UV<sub>254</sub>  
5 Aspirin® tablets, 5 Thomapyrin® tablets, 20 folded filters MN 615 1/4, 11 cm diameter, 3 sample vials 8 ml (for Aspirin sample, Thomapyrin sample, cinchona bark extract), 5 g cinchona bark, 100 ml each of chloroform, methanol, toluene/diethyl ether (55:35, v/v), spray reagent for caffeine and Dragendorff-Munier spray reagent, 50 ml each of iron(III) chloride solution and potassium hexacyanoferrate solution, 30 ml glacial acetic acid/ethyl acetate (6:2,5, v/v)  
25 ml each of 12.5% ammonia and diethylamine  
8 ml each of caffeine, paracetamol, quinine reference solutions

### TLC wine set

Chromatographic rapid test for the determination of malic, lactic, and tartaric acid in wine (evaluation of the conversion of malic to lactic acid, 2nd fermentation)

#### Contents of the TLC wine set

The kit contains 50 polyester sheets POLYGRAM® CEL 300 (4 x 8 cm) as well as all substances (cation exchanger, eluent, reference substances) and required equipment (developing chamber, capillaries, spotting guide), and a detailed instruction leaflet.



## TLC introductory kits for science education

### Ordering information

Designation	Pack of	Cat. No.
<b>TLC micro-set M (materials kit)</b>	1 kit	<b>814100</b>
<b>TLC micro-set F1</b>	1 kit	<b>814200</b>
<b>Replacement parts for TLC micro-set F1</b>		
<b>Amino acid test mixtures (for components see page 292)</b>	8 ml	<b>814201</b>
Collection of 4 individual components of the amino acid test mixture	4 x 8 ml	<b>814202</b>
<b>Cation test mixture (for components see page 292)</b>	8 ml	<b>814204</b>
Collection of 4 individual components of the cation test mixture	4 x 8 ml	<b>814205</b>
<b>TLC micro-set F2</b>	1 kit	<b>814300</b>
<b>Replacement parts for TLC micro-set F2</b>		
<b>Cholesterol reference solution</b>	8 ml	<b>814301</b>
<b>TLC micro-set F3</b>	1 kit	<b>814400</b>
<b>Replacement parts for TLC micro-set F3</b>		
<b>Quinine reference solution</b>	8 ml	<b>814405</b>
<b>Paracetamol reference solution</b>	8 ml	<b>814406</b>
<b>Caffeine reference solution</b>	8 ml	<b>814407</b>
<b>TLC wine set</b>	1 kit	<b>814500</b>
<b>Replacement parts for all TLC micro-sets</b>		
TLC polyester sheets <b>POLYGRAM® SIL G/UV<sub>254</sub></b> , 4 x 8 cm	4 x 50	<b>814025</b>
TLC polyester sheets <b>POLYGRAM® ALOX N/UV<sub>254</sub></b> , 4 x 8 cm	4 x 50	<b>814026</b>
TLC polyester sheets <b>POLYGRAM® CEL 300</b> , 4 x 8 cm	4 x 50	<b>814027</b>
TLC polyester sheets <b>POLYGRAM®</b> 4 x 8 cm: 100 x <b>SIL G/UV<sub>254</sub></b> ; 50 x <b>ALOX N/UV<sub>254</sub></b> ; 50 x <b>CEL 300</b>	1 set	<b>814028</b>
Sprühreagenzien siehe Seite 300.		

## TLC accessories

Designation	Pack of	Cat. No.
<b>Simultaneous developing chamber for TLC, 20 x 20 cm, for up to 5 plates</b>	1	<b>814019</b>
<b>Developing chambers for TLC micro-sets</b>	4	<b>814021</b>
<b>Glass laboratory sprayer with rubber bulb</b>	1	<b>814101</b>
<b>Glass capillaries 1 µl</b>	3 x 50	<b>814022</b>
<b>Rubber caps for capillaries</b>	2	<b>814102</b>
<b>Plastic syringe, 1 ml content with gradation</b>	1	<b>814104</b>
<b>Spotting guides</b>	2	<b>814023</b>
<b>Measuring cylinders, glass, 10 ml content</b>	2	<b>814024</b>
<b>Filter paper MN 713, 15 x 21 cm</b>	100	<b>814103</b>
<b>Folded filters MN 615 1/4, 11 cm Ø</b>	100	<b>531011</b>
<b>Chromatography paper MN 260, 7.5 x 17 cm (for chamber saturation)</b>	100	<b>814030</b>

**TLC simultaneous developing chamber**



20 x 20 cm, for up to 5 plates

## Adsorbents for coating TLC plates

This chapter gives a detailed description of the adsorbents manufactured by MACHEREY-NAGEL for coating of TLC plates. Especially for quantitative investigations, however, we recommend our ready-to-use layers with these adsorbents, since production of high-quality homogeneous layers for reproducible separations requires intense practical experience in coating techniques, which today is not available in many laboratories. MACHEREY-NAGEL has 35 years of experience in the coating of TLC plates and sheets.

For selection of an adsorbent one considers the properties of the compounds to be separated: first the solubility of sample compounds – including accompanying substances, which are not of interest – i.e. whether they are hydrophilic (water soluble) or hydrophobic (water insoluble). When the solution properties are evaluated, check whether they react basic, acid or neutral. Finally consider, whether the compounds can chemically react with the adsorbent or with the eluent.

## Silica gels for TLC

Silica is the most important adsorbent in thin layer chromatography. Although it can be used for most separation problems, for some very sensitive substances less active adsorbents are preferred to prevent decomposition.

Silica is a highly porous, amorphous silicic acid in the form of hard, opalescent particles, which is prepared by precipitation of water glass with sulphuric acid. Precipitation and work-up conditions determine the special properties of a silica. Specific surface area, specific pore volume, mean pore diameter etc. depend on the preparation conditions. Especially important for the reproducibility of individual silica batches is a careful control of the production conditions and subsequent grinding to a particle size suited for TLC. Changes in the particle size distribution can cause considerable variations in the separation result. MACHEREY-NAGEL can rely on long years of experience with the reproducible production of silica adsorbents.

Apart from some special cases such as buffering of a layer, pure water is used for preparation of the coating suspensions. During preparation of the suspension part of the added water is used to fill the cavity system of the silica particles. The ratio silica : water required for formation of a liquid suspension thus depends on the specific pore volume of the silica and its water content before preparation of the suspension. Thus, when the water content of different silicas is equal, the water consumption for preparation of a suspension suited for coating however different, this gives information about the difference in pore volumes. The pore volume of MN silicas is between 0.7 and 0.8 ml/g. During drying of the layers water has to be removed from the capillary system between the particles and from the cavity system of the individual silica particles. For drying an optimal temperature range between 100 and 200 °C must be observed to avoid sintering or other changes in the silica. This is the temperature where all capillary water is removed, but no water is split from the SiOH groups.

Based on these considerations we recommend the following adsorbents:

- for lipophilic substances: aluminium oxide, silica, acetylated cellulose, polyamide,
- for hydrophilic substances: cellulose, cellulose ion exchangers, kieselguhr, polyamide and the modified reversed phase silicas.

However, some of these adsorbents are only available as ready-to-use layers (see previous chapter).

In cases of doubt we recommend that in preliminary experiments you try cellulose as well as inorganic layers for solution of a certain separation problem and decide from the practical results which medium is most suited for your separation.

The characteristics of the dried layers result from the interactions of the capillary system between the grains, the actions of the cavity system in the particles and the chemical properties of the surface in the cavity system. Since neither all characteristics of the silicas nor all properties of the layer can be quantitatively described or determined, the chromatographic test on a silica layer still is very important. For this purpose the interactions of the silica layer with inorganic or organic compounds in water and non-aqueous solvents are investigated.

### Technical data of MN silicas for TLC

pore diameter	60 Å
pore volume	0.75 ml/g
specific surface (BET)	~ 500 m <sup>2</sup> /g
pH of a 10% aqueous suspension	7.0

parameter	silica grade		
	standard	high purity	preparative
particle size [µm]	2 – 20	3 – 20	5 – 50
max. iron content	0.02%	0.002%	0.02%
max. chloride content	0.02%	0.008%	0.02%

### MN silica G

The G grades contain 13% gypsum as binder and are applied successfully for the separation of numerous organic compounds. Already at the very beginning of what was later on made popular by E. Stahl as thin layer chromatography, gypsum was used as binder for enhancement of the adhesion of inorganic adsorbent layers. If today adsorbents without gypsum are prevalent, this is due to the fact that gypsum interferes with the separation of certain classes of compounds or their detection.



## Silica adsorbents for TLC

The quality of separations on layers containing gypsum is, however, in many cases better than on layers without gypsum. This fact has induced us to add gypsum even to our high purity grade Silica G-HR. Our customers have frequently confirmed that separations on layers of MN silica G HR are excellent, e.g. for **aflatoxin separations**.

For preparation of the slurry it is advantageous not to pour the whole quantity of water to the weighed amount of silica in one step, but to add it in several small portions: first stir the silica with a small amount of water to form a paste, then add the rest of water to this paste step by step.

### MN silica N

The N grades contain neither an inorganic nor an organic binder. The adhesion of the layers is sufficient; only for a few eluents slight separation of the adsorbent from the support can occur in the submerged part of the layer. Contrary to the

G grades N grades are very well suited for inorganic TLC. Prepared slurries need not be used immediately. Freshly coated plates are dried in air and activated at 110 °C for 30 minutes before use.

### High purity silica

High purity silicas (HR grades) are prepared from standard silicas by washing out inorganic impurities with acids and demineralised water. The residues of inorganic impurities (especially iron compounds) are so minute, that chromatographic separations and quantitative evaluations are no longer disturbed. Eventual organic impurities have to be removed with the respective eluent. This can be achieved by ascending chromatography prior to sample application (pre-development). The purity of the layer then depends mainly on the purity of the eluent applied.

### Ordering information

Designation	Fluorescent indicator	1 kg	5 kg
<b>Silica G</b>			
silica 60 standard grade, 13% gypsum as binder			
silica G	–	<b>816310.1</b>	<b>816310.5</b>
silica G/UV <sub>254</sub>	UV <sub>254</sub>	<b>816320.1</b>	<b>816320.5</b>
<b>Silica N</b>			
silica 60 standard grade, without binder			
silica N	–	<b>816330.1</b>	<b>816330.5</b>
silica N/UV <sub>254</sub>	UV <sub>254</sub>	<b>816340.1</b>	<b>816340.5</b>
<b>Silica G-HR</b>			
silica 60 high purity grade, gypsum as binder			
silica G-HR	–	<b>816410.1</b>	<b>816410.5</b>
<b>Silica N-HR</b>			
silica 60 high purity grade, without binder			
silica N-HR	–	<b>816430.1</b>	<b>816430.5</b>
<b>Silica P</b>			
silica, preparative grade, organic binder			
silica P/UV <sub>254</sub>	UV <sub>254</sub>	<b>816380.1</b>	<b>816380.5</b>
<b>Silica P with gypsum</b>			
silica, preparative grade, gypsum as binder			
silica P/UV <sub>254</sub> with gypsum	UV <sub>254</sub>	<b>816400.1</b>	<b>816400.5</b>

# Adsorbents for TLC

## Aluminium oxides for TLC

Aluminium oxide finds less application in TLC than silica, although it can be successfully used for adsorption chromatography, e.g. of **terpenes, alkaloids, steroids, aliphatic and aromatic compounds**. For reproducible separations it is important to control the water content of the layers, since water molecules are easily adsorbed and can block the active groups on the surface. For most separations with known relative atmospheric humidity air-dried or weakly activated layers are sufficient (e.g. 30 minutes at 75 °C or at 110 °C). By defined addition of water to the mobile phase partition chromatographic effects can be utilised for separations on aluminium oxide.

The activity of oxidic adsorbents is determined by their water content. According to Brockmann 5 activity grades are differentiated depending on their water content:

Activity grade	Water content in %	
	aluminium oxide	silica
I	–	–
II	3	10
III	6	12
IV	10	15
V	15	20

### Ordering information

Designation	Fluorescent indicator	1 kg	5 kg
<b>Aluminium oxide G</b> aluminium oxide basic with ~10% gypsum as binder, pH 7.5 – 8			
aluminium oxide G	–	<b>816010.1</b>	<b>816010.5</b>
aluminium oxide G/UV <sub>254</sub>	UV <sub>254</sub>	<b>816020.1</b>	<b>816020.5</b>
<b>Aluminium oxide N</b> aluminium oxide basic without binder, pH ~ 9			
aluminium oxide N	–	<b>816030.1</b>	<b>816030.5</b>

## Polyamide for TLC

Polyamide is often used for thin layer chromatography, because experience has shown that it is very well suited for separation of phenols and phenolic compounds. Polyamide chromatography of phenols and carboxylic acids is possible due to the reversible formation of hydrogen bonds of different strength between substances and eluents and the peptide groups of the polyamide. Eluents and substances compete for the hydrogen bonds, for this reasons compounds are suited as eluent if they can cleave the hydrogen bond between substrate and adsorbent. The elution is a displacement process: for phenols the following absolute series was found:

water < methanol < acetone < dilute sodium hydroxide solution < formamide < DMF

Aluminium oxides for chromatography are manufactured from various aluminium hydroxides by dehydration at temperatures between 400 and 500 °C. They are suited for TLC, if their BET surface is between 100 and 250 m<sup>2</sup>/g with a sufficiently large pore size (40 – 90 Å).

For coating the fine-grained oxide with a particle size below 0.06 mm is suspended with water to form a suspension of sufficient viscosity and spread onto the support. After drying in air the layer is activated at 110 °C. An aluminium oxide layer prepared as described above has an activity of II to III according to this classification.

We supply aluminium oxides for TLC with or without addition of gypsum and with or without fluorescent indicator. The pure aluminium oxides react basic (pH about 9.5). If, however, gypsum is added as binder, the pH value of an aqueous suspension is decreased to almost neutral. This is important for separations which require basic layers. Addition of gypsum as binder is indicated by the letter G and addition of a fluorescent indicator by the symbol UV<sub>254</sub>. Thus aluminium oxide G/UV<sub>254</sub> is an aluminium oxide with gypsum as binder and a fluorescent indicator with an absorption maximum of 254 nm in short-wave UV light and green fluorescence.

The high elution strength of dimethylformamide (DMF) may be due to the -CO-N groups which can form hydrogen bonds with phenolic substances in the same way as polyamide.

For separation of non-phenolic compounds partition chromatographic and other effects have to be considered, too. An understanding of the separation mechanism on polyamide is desirable, since knowledge of the separation function allows certain predictions for solution of a special separation problem on polyamide. The best chromatographic conditions can be predicted and thus a large number of time-consuming experiments can be avoided.



## Polyamide for TLC

A fundamental contribution to the practical understanding of the forces involved in polyamide chromatography was given B. Zawta and W. Hölzel [Pharmazie **23** (1968) 174 – 177, 236 – 239, 296 – 300]. They started from the following considerations:

The polyamide thin layer consists of small particles, which are however much larger than the eluent or substance molecules. On the surface and within the particles there are functional groups, i.e. peptide groups and – in much smaller number – terminal amino and carboxy groups. The eluent flows through the cavities between the particles. Between the eluent molecules and the functional groups of the polyamide different forces can appear depending on the types of molecules and groups involved. These include ionic, dipol, electron donor/electron acceptor and hydrogen donor/ hydrogen acceptor interactions. The hydrogen donor/hydrogen acceptor interactions, which lead to the formation of hydrogen bonds, play an important roll due to the nature of the functional groups on the polyamide. During chromatography the eluent molecules will first encounter the functional groups on the surface of the particles and will saturate these depending on the types of interactions involved. More eluent molecules will bind to these centres, until finally a relatively stationary liquid film is formed on the surface of the particle. In addition, molecules from this liquid film can reach the interior of the particle depending on their structure and polarity. There they can saturate more functional groups. Hydrogen bonds between the polymer chains are broken up. More eluent molecules can accumulate at these centres. The result is that the polyamide swells and under extreme condition may be dissolved. Thus, in polyamide chromatography the stationary phase consists of a liquid film on the surface and a swollen layer inside the particle, provided, naturally, that the interactions between the eluent molecules and the functional groups are not too low.

During chromatography the substances to be separated will be distributed between the resulting stationary phase and the eluent flowing between the particles – the mobile phase – ideally following the Nernst equation for partition. Simultaneously, however, substances compete in an adsorption/desorption reaction with the eluent molecules, which saturate the polyamide, for positions on the adsorbent polyamide.

One has to consider two superposing effects, which influence the separation depending on the type of eluent as well as on the type of substance to be separated. Due to the

large number of unknowns no quantitative predictions for  $R_f$  values are possible. However, the considerations mentioned above allow certain semi-quantitative information about the relative position of substances in different eluents and hence confine the number of possible eluent systems for a certain separation problem from the beginning.

The special character of the separation processes on polyamide causes some fundamental advantages compared to other adsorbents.

Due to its swelling properties polyamide has a high adsorption capacity. Thus it is suited for preparative chromatography as well as for the analysis of biological material (separation of undesirable accompanying compounds). Often polyamide chromatography is applied for the **isolation and identification of natural substances with phenolic and polyphenolic groups, e.g. anthocyanins, anthoxanthines, anthraquinone derivatives and flavones**. Via modification of adsorption and partition effects the chromatography on polyamide can be varied substantially.

Due to the medium polarity of polyamide the stationary phase (polyamide swollen with eluent) can be made more or less polar than the mobile phase. Consequently it is possible to develop in two dimensions with normal and reversed phase. Since adsorption depends not only on the number and the activity of the polar groups but also on the sterical shape of the substances, structure isomeric compounds can be well separated on polyamide.

The hydrophilic adsorbent polyamide 6 (Nylon 6 = Perlon =  $\epsilon$ -aminopolycaprolactame) is available with or without fluorescent indicator.

Finally, we want so make a few remarks about detection reactions on polyamide chromatograms. Polyamide as organic amide is not stable towards aggressive visualisation reagents (acids and bases), therefore with polyamide non-destructive detection under UV light is preferred

- viewing under short- or long-wave UV light (native fluorescence of substances to be visualised). This method is especially suited for phenolic and polyphenolic natural substances;
- fluorescence quenching (absorption) caused by the substances in question on a fluorescent plate;
- visualisation under UV light after spraying with special reagents.

### Ordering information

Designation	Fluorescent indicator	1 kg
<b>Polyamide TLC 6</b>		
$\epsilon$ -aminopolycaprolactam = nylon 6 = perlon		
polyamide TLC 6	–	<b>816610.1</b>
polyamide TLC 6 UV <sub>254</sub>	UV <sub>254</sub>	<b>816620.1</b>

## Cellulose powders for TLC

### Unmodified cellulose

Thin layer chromatography on cellulose can be considered as an extension of classical paper chromatography. The separation is mainly based on partition chromatographic mechanisms, however, adsorption effects cannot be excluded. The native, fibrous cellulose MN 301 replaces our successful cellulose MN 300.

**Analytical data of important cellulose powders**

Parameter	MN 301	MN 301 A	MN 301 HR
residue on ignition at 850 °C	≤ 1500 ppm	≤ 500 ppm	≤ 200 ppm
iron as Fe	≤ 20 ppm	≤ 20 ppm	2 ppm
copper as Cu	6 ppm	≤ 6 ppm	1 ppm
phosphate as P	7 ppm	≤ 7 ppm	–
methylene chloride extract	≤ 0.25%	≤ 0.01%	≤ 0.025%
average degree of polymerisation	400 – 500	400 – 500	400 – 500
fibre length (95%)	2 – 20 µm	2 – 20 µm	2 – 20 µm
specific surface acc. to Blaine	15000 cm <sup>2</sup> /g	15000 cm <sup>2</sup> /g	15000 cm <sup>2</sup> /g

### Native fibrous cellulose MN 301

Fibrous cellulose MN 301 is a high quality cellulose, which is prepared according to the standard processes of the cellulose-manufacturing industry and is subsequently ground under the mildest conditions to the fine fibre size required for TLC. The average degree of polymerisation (DP) of native cellulose powder MN 301 is in the range of about 400 – 500, the fibres of MN 301 are about 2 – 20 µm long.

An aqueous suspension with about 25% cellulose powder – homogenised 30 to 60 seconds in an electric mixer – produces layers with optimal homogeneity, smoothness etc. The adherence of these layers is excellent; when dry they are rub-fast. Cellulose layers are best dried in air. Activation of cellulose layers at elevated temperatures is not necessary, rather detrimental. Longer storage of cellulose layers in air on the contrary even enhances their separation performance. Cellulose powder MN 301 differs substantially from chromatography paper by the fine fibres, those in paper being long and well-preserved. The short fibres of cellulose powders prevent the almost instantaneous spreading of substances, which occurs along the boundaries of long fibres. Thus substance spots of equal concentration are more compact than on paper. The high specific surface of MN 301 causes a larger substance capacity which again results in compacter spots than on paper.

### High purity fibrous cellulose MN 301 HR

Cellulose powder MN 301 HR is acid-washed under very mild conditions and after neutral washing freed from fat and resins with organic solvents. Hydrolysis of the cellulose is avoided as far as possible, thus the adhesive properties of the starting cellulose MN 301 are maintained. A slight yellow front does not occur and spots are completely compact and "branchless" even up to  $R_f$  values of 1.0. High purity cellu-

lose powder is especially recommended for quantitative investigations, e.g. for **separation of carbohydrates** with consequent IR spectroscopy as well as for **separation of phosphoric acids, phosphates** etc.

### Cellulose MN 301 A for the <sup>32</sup>P postlabelling procedure

Cellulose MN 301 A is a variant of our cellulose MN 301, which has been developed for the <sup>32</sup>P postlabelling procedure. A special treatment eliminates all lactobacilli contaminations of the cellulose. This cellulose is **not** impregnated with PEI, but designed for impregnation and coating by the user.

### Microcrystalline cellulose AVICEL®

Microcrystalline cellulose AVICEL® is only available as ready-to-use layers MN CEL 400 (see pages 279).

### Coating of cellulose powders

With fibrous cellulose powder MN 301 gel formation is only noticeable after homogenisation of more than 10 minutes. With MN 301 it is not possible to obtain a homogenisation sufficient for coating by simply stirring with a glass rod – except if organic solvents are used. Adhesive additives are not necessary with MN cellulose powders. The adherence of layers is several times higher than with inorganic adsorbents; when dry they are rub-fast. The thickness of dry cellulose layers never corresponds to the thickness set on the coating instrument. As a rule during drying cellulose layers shrink towards about half the width set on the instrument. Cellulose layers of about 125 µm (when dry) are recommended for most separations and demonstrate most obviously the advantages of thin layer chromatography. Thicker cellulose layers can be prepared from fibrous cellulose powder MN 301 or MN 301 HR up to 0.5 mm when dry without formation of cracks.

### Cellulose ion exchangers

In thin layer chromatography powdered cellulose ion exchangers are mainly used for **separation of proteins, amino acids, enzymes, nucleic acids, hormones and viruses**. The most used for these applications is DEAE cellulose.

Cellulose ion exchangers possess ion exchanging properties like the resin exchangers, however, they differ in some important points. Ion exchange celluloses are prepared from native cellulose by bonding basic groups via ether or ester bridges. The large surface area of the cellulose exchanger, which results from the fibre structure, causes a large number of substituents to be close to the surface. This is why even large hydrophilic molecules like e.g. proteins can easily penetrate the swollen hydrophilic cellulose matrix. With exchange resins this penetration is not possible due to the hydrophobic characteristics of the synthetic resin matrix, so that in these resins only the active groups on the spherical surface can participate in the reaction. The majority of active groups of exchange resins are, however, located inside the resin matrix.

The distances of the active groups are about 50 Å for cellulose exchangers, which is very long compared to the distance of about 10 Å for exchanger resins. The result is that cellulose exchangers, despite their numerically much smaller exchange capacity, have a larger capacity than resin exchangers for large molecules such as proteins. In addition, due to the much longer distances between active groups,



## Cellulose powders for TLC

bonding only occurs at one or very few positions, enabling a selective desorption under very mild conditions as compared to exchange resins. Last but not least, due to these characteristics cellulose ion exchangers are very valuable tools for the **separation, purification and isolation of labile substances in biochemistry**.

*Diethylaminoethyl (DEAE) cellulose R-O-C<sub>2</sub>H<sub>4</sub>-N(C<sub>2</sub>H<sub>5</sub>)<sub>2</sub>*

DEAE cellulose – prepared by reaction of alkal cellulose with 2-chloro-1-diethylaminoethane hydrochloride – is a relatively strong basic anion exchanger. The diethylaminoethyl group which is bonded to the cellulose via an ether group causes a base strength comparable to that of exchange resins. The basic group is monovalent. Our DEAE cellulose has an exchange capacity of about 0.35 meq/g.

DEAE cellulose is the most frequently used cellulose ion exchanger. It is mainly used for **separation, purification and isolation of proteins, enzymes and hormones**.

*Preparation of cellulose ion exchange layers*

Layers of cellulose ion exchangers are prepared like layers of other adsorbents. About 10 – 20% cellulose powder are homogenised in distilled water. The quantity of powder depends on the swelling properties of the exchanger. Heavy swelling is

disadvantageous since after complete drying the layer will show more or less fine cracks. This is especially true for thick exchanger layers; however, it can be prevented by adding a small percentage of unmodified cellulose MN 301. Freshly prepared exchanger layers are always dried in air. Activation in a drying cabinet at elevated temperatures is not necessary. For a good reproducibility of separations it is important that possible impurities from the exchanger be removed and that the active groups are present in the most exchange-active form. Predevelopment with distilled water is recommended.

The following eluents are recommended for cellulose ion exchangers:

- buffer solutions of different ionic strengths in the pH range 3 to 8, e.g. 0.005 M phosphate buffer pH 8 or 2 M sodium formate buffer pH 3.4,
- salt solutions such as 0.1 – 2 M LiCl or NaCl solution,
- distilled water.

Cellulose ion exchangers for TLC are used for the **separation of nucleic acid constituents and nucleotides, coenzymes, steroid sulphates, proteins, phosphate esters, sugar phosphates, vitamins, amino acids** and other compounds.

### Ordering information

Designation	Comments	Fluorescent indicator	1 kg	5 kg
<b>Cellulose MN 301</b> native fibrous cellulose, unmodified				
cellulose MN 301		–	<b>816250.1</b>	<b>816250.5</b>
cellulose MN 301 UV <sub>254</sub>		UV <sub>254</sub>	<b>816260.1</b>	<b>816260.5</b>
cellulose MN 301 HR	acid-washed and defatted	–	<b>816270.1</b>	<b>816270.5</b>
cellulose MN 301 A	for the <sup>32</sup> P postlabelling procedure	–	<b>816300.1</b>	<b>816300.5</b>
<b>Cellulose MN 300 DEAE</b>				
diethylaminoethyl cellulose, ion exchanger for TLC, exchange capacity ~ 0.35 mval/g				
Cellulose MN 300 DEAE		–	<b>816210.1</b>	

## Fluorescent indicators

In order to take advantage of all possibilities of TLC it is important to use fluorescent indicators with efficient radiation, which allow evaluation of chromatograms in the short-wave as well as in the long-wave UV range. We supply two UV fluorescent indicators, namely the types UV<sub>254</sub> and UV<sub>366</sub>, as pure substances or added to adsorbents. Most of our ready-to-use layers are also available with or without fluorescent indicator. Our fluorescent indicator UV<sub>254</sub> is a manganese activated zinc silicate with the absorption maximum

at 254 nm. It shows a green fluorescence. The fluorescent indicator UV<sub>366</sub> is an inorganic fluorescent pigment as well, however, with an absorption maximum at 366 nm. It shows a blue fluorescence. It should be noted that the fluorescent indicator UV<sub>254</sub> is relatively susceptible towards acids and that its fluorescence can be completely quenched by acidic solvents. Please note, that for our Nano TLC plates we use another UV indicator with absorption maximum at 254 nm, which is acid-resistant.

### Ordering information

Composition	Absorption maximum	Colour of fluorescence	100 g
<b>Fluorescent indicator UV<sub>254</sub></b>			
manganese-activated zinc silicate	254 nm	green	<b>816710.01</b>
<b>Fluorescent indicator UV<sub>366</sub></b>			
inorganic fluorescent pigment	366 nm	blue	<b>816720.01</b>

# Visualisation reagents

## MN programme of spray reagents for TLC

### Ordering information

Description	Solvent	Application	Pack of	Cat. No.
Aniline phthalate spray reagent	2-propanol	reducing sugars, oxohalic acids	100 ml	<b>814919</b>
Bromocresol green spray reagent	2-propanol	organic acids	100 ml	<b>814920</b>
Caffeine spray reagent	water/acetone	caffeine	100 ml	<b>814401</b>
2',7'-Dichlorofluorescein spray reagent	2-propanol	lipids (saturated, unsaturated)	100 ml	<b>814921</b>
4-(Dimethylamino)-benzaldehyde spray reagent	2-propanol	terpenes, sugars, steroids	100 ml	<b>814922</b>
Dragendorff-Munier spray reagent	water	alkaloids and other nitrogen compounds	100 ml	<b>814402</b>
Iron(III) chloride solution	water	acetylsalicylic acid, paracetamol	100 ml	<b>814403</b>
Potassium hexacyanoferrate(III) solution	water		100 ml	<b>814404</b>
Molybdatophosphoric acid spray reagent	ethanol	lipids, sterins, steroids, reducing compounds	100 ml	<b>814302</b>
Ninhydrin spray reagent	ethanol	amino acids, amines and amino sugars	100 ml	<b>814203</b>
Rhodamin B spray reagent	ethanol	lipids	100 ml	<b>814923</b>
Rubeanic acid spray reagent	ethanol	heavy metal cations	100 ml	<b>814206</b>

## Frequently used visualisation reagents for TLC

This chapter describes some frequently used TLC visualisation reagents in alphabetical order. For information as to which reagents are suited for which classes of compounds please refer to the chapter "TLC applications" of our separate TLC catalogue. Although in most cases we talk about spray reagents, the same reagent solutions are of course applicable for dipping TLC plates. Older papers often cite benzene which in many cases can be substituted by the less toxic toluene. For further information please refer to the following literature:

Jork, Funk, Fischer, Wimmer, *Dünnschicht-Chromatographie, Reagenzien und Nachweismethoden, Band 1 a, Physikalische und chemische Nachweismethoden: Grundlagen, Reagenzien I*, VCH Verlagsgesellschaft, Weinheim (FRG) 1989

E. Stahl, *Dünnschicht-Chromatographie, 2. Auflage*, Springer Verlag Berlin, 1967; *Thin Layer Chromatography, 2nd Edition*, Reprint 1988

K. Randerath, *Dünnschicht-Chromatographie*, Verlag Chemie 1965

### 4-Aminoantipyrine/potassium hexacyanoferrate(III)

(Emerson reagent) for detection of phenols and aryl amines

#### Solutions:

- 1 g aminoantipyrine (4-aminophenazone) in 100 ml 80% ethanol
2. 4 g potassium hexacyanoferrate(III) in 50 ml water, fill up to 100 ml with ethanol

#### Procedure:

- spray with solution 1
- dry 5 minutes with warm air
- spray with solution 2
- again dry 5 minutes with warm air
- place chromatogram in a chamber with vapour from 25% ammonia solution, making sure that the layer does not contact the liquid

**2-Aminoethyl diphenylborate** see under Ethanolamine diphenylborate

**Ammonium metavanadate, ammonium monovanadate** see under Vanadium(V)/sulphuric acid reagent

**Ammonium molybdate** for detection of phosphoric acid derivatives

#### Solutions:

1. 1 M perchloric acid in water/acetone (1:1)
2. ammonium molybdate solution: 5 g  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$  in 35 ml semi-concentrated nitric acid and 65 ml water
3. tin(II) chloride solution: 0.5 g  $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$  in 100 ml 0.5 M hydrochloric acid

#### Procedure:

- dry developed chromatogram and heat to 60 °C
- hydrolyse di- and triphosphates by spraying perchloric acid (solution 1) onto the warm plate. After spraying 2 times, dry plate slowly at 50 °C. Amidophosphates e.g. need not be decomposed.
- in any case spray the still warm plate with ammonium molybdate solution (solution 2)
- then spray the still wet plate with tin(II) chloride solution (solution 3). Phosphates appear as blue to blue-green spots.

Polyphosphates can also be detected by dipping the plates in a solution of ammonium molybdate (1 g) dissolved in water (8 ml) and perchloric acid (3 ml, ca. 70%), filled up to 100 ml with acetone. Then phosphates appear as yellow-green spots on a blue background. Also see Molybdenum blue reaction according to Dittmer and Lester



## Frequently used visualisation reagents for TLC

**Aniline phthalate spray reagent** for detection of reducing sugars

*Procedure:*

- dry the developed chromatogram
- spray with aniline phthalate spray reagent
- briefly dry with hot air
- heat to 80 – 130 °C for 20 to 30 min

Resulting substance spots show different colours on an almost colourless background. Some spots give a fluorescence at 365 nm.

**Anisidine phthalate reagent** for detection of carbohydrates

- spray with a solution of 1.23 g *p*-anisidine and 1.66 g phthalic acid in 100 ml 95% ethanol

Hexoses yield green, methylpentoses yellow-green, pentoses purple, uronic acids brown spots.

**Boute reaction** for detection of phenols

*Procedure:*

- dry and heat the developed chromatogram
- place hot plate for 3 – 10 minutes in a chamber with NO<sub>2</sub> vapour (from conc. nitric acid)
- then treat with NH<sub>3</sub> vapour (from conc. ammonia)

**Bromine/carbon tetrachloride** for detection of organothiophosphorus pesticides

- place chromatogram in a chamber with 10% bromine/carbon tetrachloride without contact of the liquid

**Bromocresol green** for detection of acids

- dip chromatogram in a solution of 0.1 g bromocresol green in 500 ml ethanol and 5 ml 0.1 M sodium hydroxide solution

Acids yield yellow spots on blue background

**Chloranil reagent** for detection of phenols

- spray with a solution of 1% tetrachloro-*p*-benzoquinone in benzene or toluene

**Chlorine/o-tolidine** for detection of compounds forming chloroamines, e.g. urea derivatives, carbamates, antibiotics

*Solutions:*

1. 160 mg *o*-tolidine in 30 ml glacial acetic acid, filled up to 500 ml with dist. water, plus 1 g potassium iodide
2. saturated solution of *o*-tolidine in 2% acetic acid/0.85% potassium iodide solution (1:1, v/v)

*Procedure A:*

- place chromatogram 15 – 20 minutes in a chlorine atmosphere (e.g. 1.5% potassium permanganate + 10% hydrochloric acid)
- leave 5 minutes at ambient temperature until the chlorine is evaporated completely
- spray with solution 1

*Procedure B (according to Greig and Leback)*

- spray with 2% potassium hypochlorite solution in water
- leave 1 – 1.5 hours at ambient temperature
- spray with solution 2

**DDQ reagent (dichlorodicyanobenzoquinone)** for detection of phenols

- spray with a solution of 2% 2,3-dichloro-5,6-dicyano-1,4-benzoquinone in benzene or toluene

**Dichlorofluorescein reagent** for detection of sweeteners saccharin and cyclamate

- spray with a 0.2% solution of dichlorofluorescein in 96% ethanol
- dry with warm air
- if necessary spray with water
- view under UV at 360 nm

**Dichlorofluorescein/fluorescein sodium salt** for detection of N-substituted barbiturates

- spray with a 0.1% ethanolic solution of dichlorofluorescein
- then spray with a 0.1% ethanolic solution of the fluorescein sodium salt

**2,6-Dichloroquinone-4-chloroimide** for detection of antioxidants, phenols, primary and secondary aliphatic amines, secondary and tertiary aromatic amines, aromatic hydrocarbons, pharmaceuticals, phenoxyacetic acid herbicides etc.

- spray with a freshly prepared 0.5 – 2% solution of 2,6-dichloroquinone-4-chloroimide in ethanol
- heat 10 minutes to 110 °C
- treat with ammonia vapour

***p*-Dimethylaminobenzaldehyde / hydrochloric acid reagent (Ehrlich's reagent)** for detection of amines, indole derivatives

- spray with a solution of 1% *p*-dimethylaminobenzaldehyde in conc. hydrochloric acid/methanol (1:1)
- heat plates 20 min to 50 °C

**Dithizone** for detection of metal ions

*Solution:*

Dissolve 20 mg dithizone in 100 ml acetone, store in a brown bottle in a refrigerator.

*Procedure:*

- spray with dithizone solution
- spray with 25% ammonia solution

**Dittmer and Lester** see Molybdenum blue reaction

**Dragendorff reagent** for detection of nitrogen compounds, alkaloids, antiarrhythmic drugs, surfactants

*Solutions:*

1. 1.7 g basic bismuth nitrate and 20 g tartaric acid in 80 ml water
2. 16 g potassium iodide in 40 ml water

*Stock solution (stable several weeks in a refrigerator):* mix equal volumes of solutions 1 and 2

*Procedure:*

- spray with a solution of 10 g tartaric acid, 50 ml water and 5 ml stock solution

## Frequently used visualisation reagents for TLC

**Ethanolamine diphenylborate** – (flavone reagent according to Neu), e.g. for flavonoids

- spray with a 1% solution of ethanolamine diphenylborate in methanol
- spray with a 5% ethanolic solution of polyethylene glycol for fluorescence stabilisation
- irradiate 2 minutes with intense UV (365 nm)
- view under UV at 365 nm

**Ehrlich's reagent** see *p*-Dimethylaminobenzaldehyde

**Emerson reagent** see 4-Aminoantipyrine/potassium hexacyanoferrate(III)

**Fast Blue B reagent** for detection of cannabinoids, phenols, tanning agents, amines which can be coupled

- spray with a solution of 0.5 g Fast Blue B [tetraazotised di-*o*-anisidine] in acetone/water (9:1, v/v), always prepared fresh
- spray with 0.1 M sodium hydroxide solution

**Flavone reagent acc. to Neu** see under Ethanolamine diphenylborate

**Fluorescamine** for detection of primary and secondary amines, peptides and sulphonamides, e.g. nitrosamines after photolysis

- spray plate with a solution of 0.1 mg/ml 4-phenylspiro[furan-2(3H),1-phthalan]-3,3-dione in acetone, prepared fresh daily
- for stabilisation of fluorescence at 366 nm spray with 10 g triethylamine, filled up to 100 ml with dichloromethane

**Formaldehyde/sulphuric acid** for detection of alkaloids, aromatic hydrocarbons, e.g. antihypertensive drugs

- spray with a solution of 37% formaldehyde in conc. sulphuric acid (1:10)

**Gibb's reagent** for detection of phenols. For further applications see 2,6-Dichloroquinone 4-chloroimide.

- spray with a solution of 2% 2,6-dibromo-*N*-chloro-*p*-benzoquinone imine in benzene or methanol

**Hydroxylamine/iron(III) chloride** for detection of amides, lactones, carboxylic acid esters and anhydrides

*Solutions:*

1. mix 1 vol. part of 7 g hydroxylammonium chloride in 100 ml methanol with 1 vol. part of a solution of 7.2 g potassium hydroxide in 100 ml methanol. Filter from precipitated potassium chloride.
2. 2% solution of iron(III) chloride in 1% aqueous hydrochloric acid

*Procedure:*

- spray airdried plate first with solution 1, then with solution 2.

**Iodine compounds** – detection by decomposition under UV

- dry plates at 100 °C
- after cooling spray with little 50% acetic acid
- irradiate some minutes with unfiltered UV light

Iodine compounds show weakly violet to brown spots. The colour can be enhanced by spraying to transparency with 10% acetic acid and irradiation with UV light (sudden appearance of blue spots)

**Iodine vapour**, relatively unspecific universal reagent for many organic compounds

- charge chamber with some crystals of iodine
- place developed, dried chromatogram in I<sub>2</sub> atmosphere

**Iodoplatinate reagent** for detection of organic nitrogen compounds, alkaloids, e.g. cocaine metabolites

- spray with a freshly prepared mixture of 3 ml hexachloroplatinic(IV) acid solution (10%) in 97 ml water and 100 ml 6% aqueous potassium iodide solution

**Iron(III) chloride / potassium hexacyanoferrate / sodium arsenate (according to Patterson and Clements)** for detection of iodine compounds, e.g. thyroid gland hormones

*Solutions:*

1. 2.7% iron(III) chloride hexahydrate in 2 N hydrochloric acid
2. 3.5% potassium hexacyanoferrate in water
3. dissolve 3.8 g arsenic trioxide in 25 ml 2 N sodium hydroxide solution heating slightly, cool to 5 °C and add 50 ml 2 N sulphuric acid, fill up to 100 ml with water

*Procedure:*

- immediately before use mix 5 ml solution 1, 5 ml solution 2 and 1 ml solution 3; spray onto the dry layer
- dry carefully (temperature below 50 °C)
- cover with glass plate and leave 15 minutes in the dark

**Lead tetraacetate/2,7-dichlorofluorescein** for detection of vicinal diols, glycosides and phenols, e.g. sugar acids

*Solutions:*

1. 2% (w/v) lead tetraacetate in glacial acetic acid
2. 1% (w/v) 2,7-dichlorofluorescein in ethanol

Mix 5 ml each of solution 1 and 2 and fill up to 200 ml with dry benzene or toluene. This reagent solution is stable for only about 2 hours.

**Manganese/salicylaldehyde** for detection of organothio-phosphorus pesticides

*Solutions:*

1. dissolve 100 mg manganese chloride (MnCl<sub>2</sub> · 4 H<sub>2</sub>O) in 100 ml 80% ethanol
2. dissolve 1.3 g 2-hydrazine quinoline in the lowest possible volume of hot ethanol. Dissolve 1 g salicylaldehyde in 5 ml ethanol and add 1 – 2 drops glacial acetic acid. Combine both solutions and reflux 30 min. The crystals of salicyl-2-aldehyde 2-quinolyldiazine precipitated during cooling are recrystallised from ethanol. For solution 2 dissolve 50 mg of the salicyl derivative in 100 ml ethanol.



## Frequently used visualisation reagents for TLC

### Procedure:

- spray with a mixture of equal volumes of solutions 1 and 2

**Mandelin's reagent** see under Vanadium(V)/sulphuric acid reagent

**Mercury(II) chloride/dithizone** for detection of barbiturates

- spray with a freshly prepared 1:1 mixture of 1 – 2% mercury(II) chloride in ethanol and 0.1 – 0.2% dithizone in ethanol
- view under UV light at 360 nm

**4-Methoxybenzaldehyde/sulphuric acid/ethanol** for detection of erythromycin and metabolites

- spray with 4-methoxybenzaldehyde/sulphuric acid/ethanol (1:1:9, v/v/v)
- heat 1 min to 110 °C

**Methyl yellow** for detection of chlorinated insecticides and antimicrobial compounds

### Solution:

dissolve 0.1 g methyl yellow (N,N-dimethyl-4-phenylazo-aniline) in 70 ml ethanol, add 25 ml water and fill up to 100 ml with ethanol

### Procedure:

- spray with reagent solution
- dry at ambient temperature
- irradiate 5 min with UV light (without filter)

**Molybdatophosphoric acid** for detection of reducing substances, e.g. alcohols, bile acids, lipids, fatty acids, steroids

- spray with a solution of 250 mg molybdatophosphoric acid in 50 ml ethanol
- heat to 120 °C until spots appear (heatgun)
- if necessary treat with ammonia vapour

The reagent solution is stable for only about 10 days even in the dark.

**Molybdenum blue reaction** according to Dittmer and Lester for detection of phospholipids and phosphoric acid derivatives

### Solutions:

1. boil 40.11 g MoO<sub>3</sub> in 1 l 25 N sulphuric acid for 3 – 4 hours until the molybdenum oxide is completely dissolved. Let the light yellow solution slowly cool to ambient temperature over night, the solution will turn light blue.
2. boil 1.78 g molybdenum powder and 500 ml of solution 1 for 15 min, cool and decant from the remaining residue.

For preparation of the spray reagent add equal volumes of solutions 1 and 2 to 4.5 volume parts water. A dark green solution is formed.

Solutions 1 and 2 are stable for several months when stored in the dark, the spray reagent has to be prepared about weekly.

**Ninhydrin** for detection of amino acids, amines, aminosugars

- spray with a solution of 0.2 g ninhydrin in 100 ml ethanol
- heat to 110°C until reddish spots appear

**Ninhydrin/cadmium acetate** for detection of amino acids, and heterocyclic amines

- dissolve 1 g ninhydrin and 2.5 g cadmium acetate in 10 ml glacial acetic acid and fill up to 500 ml with ethanol
- spray and heat 20 minutes to 120 °C

**Ninhydrin/pyridine/glacial acetic acid** for detection of peptides

- spray with 1% ninhydrin in pyridine / glacial acetic acid (5:1, v/v)
- heat 5 minutes to 100 °C

**Nitric acid/ethanol** for detection of amines, and alkaloids

- spray with a solution of 50 drops 65% nitric acid in 100 ml ethanol (higher acid concentrations are also possible)
- if necessary heat to 120 °C for some time

**Patterson and Clements** see under Iron(III) chloride/potassium hexacyanoferrate/sodium arsenate

**Phenylhydrazine sulphonate** for detection of some antimicrobial compounds

### Solutions:

1. dissolve 3.5 g phenylhydrazine 4-sulphonic acid hemihydrate in 10 ml water and 20 ml 1 N sodium hydroxide solution
2. mix 30 ml 2 N sodium hydroxide solution with 40 ml acetone

The spray reagents have to be prepared fresh every time.

### Procedure:

- wet chromatogram evenly with spray solution 1
- after air drying the plate shake spray solution 2 and spray

**Phosphomolybdic acid** see under Molybdatophosphoric acid

**Pinacryptol yellow** for detection of sweeteners, surfactants

### Solution:

Dissolve 100 mg pinacryptol yellow in 100 ml hot water or ethanol

### Procedure:

- spray with reagent solution
- view under UV light

**Potassium dichromate/sulphuric acid** (chromosulphuric acid), universal visualisation reagent for organic compounds (e.g. alcohols, also for bile acids, lipids)

- spray with a solution of 5 g potassium dichromate in 100 ml conc. sulphuric acid
- if necessary heat plate to 150 °C

## Frequently used visualisation reagents for TLC

**Potassium permanganate/sulphuric acid**, universal reagent for organic compounds, e.g. fatty acid derivatives

- spray with a solution of 1.6% potassium permanganate in conc. sulphuric acid (Beware of explosions during dissolution!)
- heat plates 15 to 20 minutes to 180 °C

**Pyrocatechol violet reagent** for detection of organotin compounds

- completely decompose metalorganic compounds by irradiation of the developed plates with UV light
- dip plates in a solution of 1 g pyrocatechol violet in 1000 ml ethanol (99.5%)

**Rhodamin B spray reagent** for detection of lipids

*Procedure:*

- dry the developed chromatogram
- spray with rhodamin B spray reagent

In most cases red-violet zones with an intense fluorescence at 365 nm develop on a pink background.

RP phases are less suited, because in this case the environment of the spots also forms an intense colour. By placing the sprayed chromatograms into an NH<sub>3</sub> atmosphere the detection sensitivity can be improved.

**Rubeanic acid** for detection of heavy metal ions

- spray with a solution of 0.5% rubeanic acid in ethanol
- dry briefly
- spray with 25% ammonia solution or place in a chamber with ammonia vapour

**Silver nitrate/hydrogen peroxide** for detection of halogenated hydrocarbons

*Solution:*

Dissolve 0.1 g silver nitrate in 1 ml water, add 10 ml 2-phenoxyethanol, fill up to 200 ml with acetone and add 1 drop hydrogen peroxide (30% solution)

*Procedure:*

- spray plate with reagent solution
- irradiate with unfiltered UV light until optimal contrast is obtained

Luckow [Fresenius Z. Anal. Chem. **294** (1979) 288] uses silver nitrate/phenoxyethanol/acetone without addition of hydrogen peroxide for detection of the pesticide ioxynil (3,5-diiodo-4-hydroxybenzonitrile).

**Sodium nitroprussiate/potassium hexacyanoferrate(III)** for detection of aliphatic nitrogen compounds

*Solution:*

Mix 1 vol. part each of 10% aqueous sodium hydroxide solution, 10% sodium nitroprussiate solution and 10% potassium hexacyanoferrate(III) solution with 3 vol. parts water. Let the solution stand at least 20 min at ambient temperature before use. Stored in a refrigerator, it is stable for several weeks.

*Procedure:*

- for use mix the reagent solution with an equal volume of acetone and spray

**TCNE reagent (tetracyanoethylene)** for detection of aromatic hydrocarbons and heterocycles, aromatic amines and phenols

- spray with a solution of 0.5 – 1 g tetracyanoethylene in dichloromethane or benzene

**TNF reagent (trinitrofluorenone)** for detection of phenols

- spray with a solution of 2% 2,4,7-trinitrofluorenone in benzene or toluene

**o-Tolidine/diazotised** for detection of phenols

*Solutions:*

- tolidine solution: fill up 5 g o-tolidine and 14 ml conc. hydrochloric acid to 100 ml with water
- nitrite solution: 10% aqueous sodium nitrite solution, prepared fresh
- spray solution: mix 20 ml tolidine solution and 20 ml nitrite solution at 0 °C, stirring constantly. The spray solution is stable for about 2 - 3 hours.

After spraying it can take several hours until coloured spots are formed!

**Triethanolamine**, 20% in isopropanol for enhancement of fluorescence

**Vanadium(V)/sulphuric acid reagent** for detection of carbohydrates, glycols, reducing carboxylic acids, steroids, antioxidants, vitamins, phenols, aromatic amines

- Ammonium monovanadate (ammonium metavanadate)/sulphuric acid reagent
  - spray with a solution of 1.2 g ammonium monovanadate in 95 ml water + 5 ml conc. sulphuric acid or
  - for  $\beta$ -blockers: spray with a saturated solution of ammonium monovanadate in conc. sulphuric acid
- Vanadium pentoxide/sulphuric acid reagent
  - Spray with a solution of 1.82 g vanadium pentoxide in 30 ml 1 M sodium carbonate, sonicate to achieve complete dissolution, after cooling add 46 ml 2.5 M sulphuric acid and fill up to 100 ml with acetonitrile

**Vanillin reagent** for detection of amines and amino acids

- spray with a solution of 1 g vanillin in 50 ml 2-propanol
- dry 10 minutes at 110 °C
- spray with 1 ml 1 M potassium hydroxide solution, filled up to 100 ml with ethanol
- dry 10 minutes at 110 °C
- view under UV at 365 nm

**Vanillin/sulphuric acid reagent** for detection of steroids

- spray with a 1% solution of vanillin in conc. sulphuric acid
- heat 5 minutes to 105 °C