

# Staining in Thin-Layer Chromatography



**MERCK**

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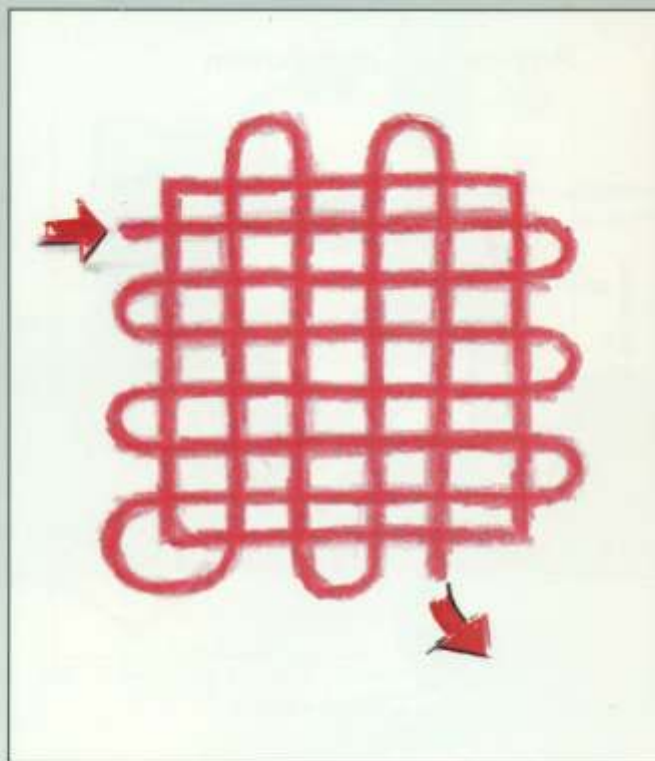
## Staining in Thin-Layer Chromatography

It is necessary to be able to recognize the separated substances in order to evaluate a thin-layer chromatogram. Substances that are intrinsically coloured or fluoresce under UV light can be detected by inspection in visible light or under short- or long-wavelength UV light. The use of precoated layers with fluorescence indicators ( $F_{254}$ ) allows the detection of substances that absorb short-wavelength UV light ( $\lambda = 254 \text{ nm}$ ). They appear as dark chromatogram zones on a green fluorescent background. Compounds that are not intrinsically coloured, do not absorb UV light and cannot be stimulated to fluorescence must be reacted with a suitable reagent to make them visible. The most frequent technique employed in this situation is to spray the chromatogram. Here a proper choice of spray reagents can increase the specificity of detection, i.e. make it possible to assign a compound to a particular substance class. The sensitivity of detection is often increased.

Optimum staining of a TLC plate requires that the spray solution be very finely divided and evenly distributed, i.e. the spray solution is best applied to the plate in a meandering pattern in the form of an aerosol. It is usually necessary to heat the stained

chromatogram in order to bring about rapid and complete derivatization. Drying cupboards, hot plates and IR sources are suitable for this purpose. In general the reagent should be sprayed in an efficient fume hood.

In order to prevent damage to the ozone layer in the atmosphere Merck replaced normal FCH-containing aerosol spray cans with ready-to-use, propellant-free spray solutions a long time ago. In order to round off this environmentally friendly product line and to achieve optimum and reproducible spray mist quality the range is being completed with a very simple to use spray unit that is very suitable for TLC.



Recommended spray scheme



Spraying a TLC plate with the TLC-sprayer



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## The TLC-sprayer

The TLC-sprayer is an electropneumatically operated spray system. This atomizes the liquid being sprayed with pressurized air supplied by a rotary compressor in a two-component insert via a mechanical break-up nozzle. This brings about proper aerosol formation with particle-size distribution in the range from 0.3 to 10  $\mu\text{m}$ . In contrast hydromechanical spray systems without two-component nozzles deliver a markedly wet spectrum, i.e. it is primarily larger diameter particles that are delivered to the layer. Proper aerosol spraying is advantageous in TLC: it provides uniform wetting and distribution of the reagent on the TLC layer while minimizing consumption, thus providing for an optimum utilization.

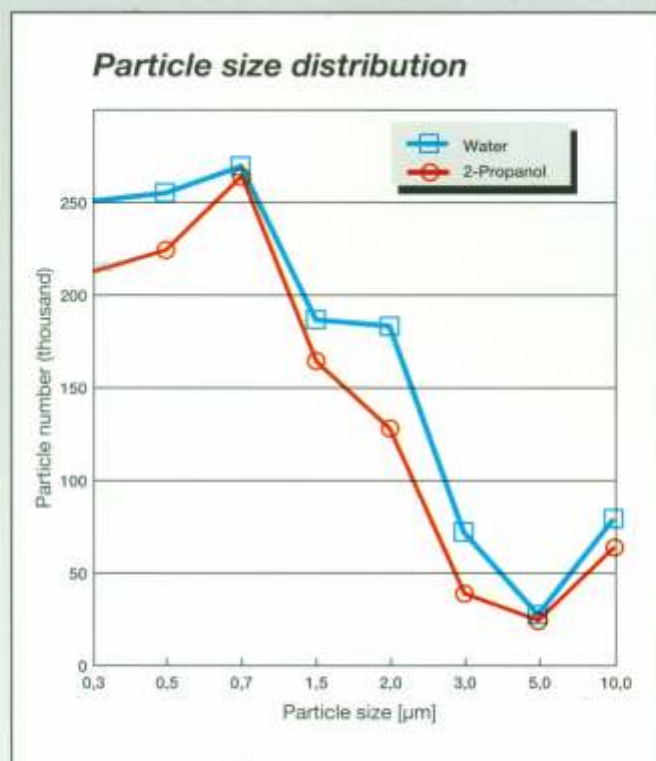
The basic equipment of the TLC-sprayer comprises, together with a charger and spray holder, two spray heads the diameters of whose capillaries differ from each other. A spray head with a smaller cross section (0.8 mm) is used for spraying alcoholic reagent solutions. The spray head with the larger diameter reagent capillaries (1.25 mm) is used for more viscous solutions, such as

for example sulphuric acid reagents. It can also be used to spray low viscosity solutions at higher rates with approximately equal spray results.

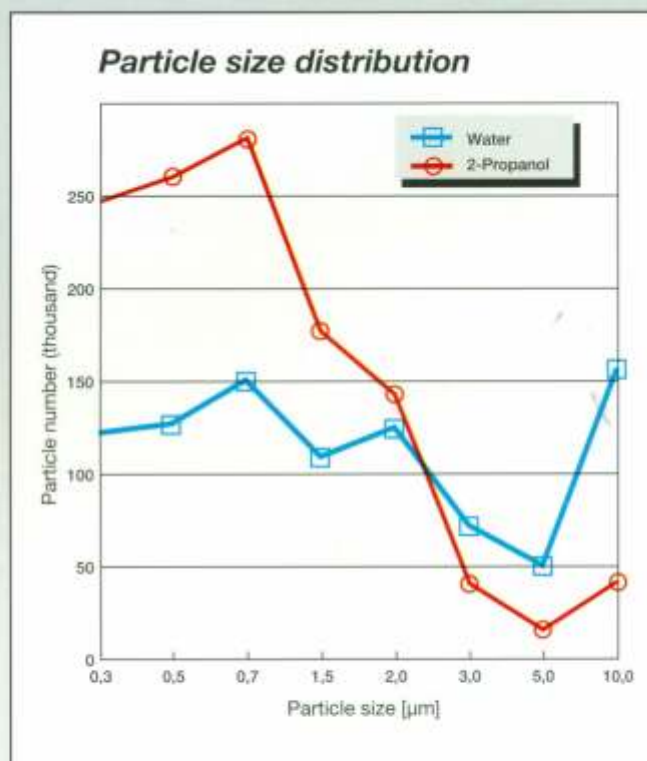
## Ordering information

Order No.	Description
8540.0001	TLC-sprayer*

\* comprising: charger, spray holder, 2 reagent bottles and 2 spray heads with different diameters of reagent capillaries.



Spray head 0.8 mm (spray rate: 0.14 g/s)



Spray head 1.25 mm (spray rate: 0.17 g/s)

## Technical details

Charger	
Mains connection:	100–240 V / 50–60 Hz
Power consumption:	ca. 5 W / h
Spray holder	
Operating voltage:	4.8 V DC
Inductive charging:	45–50 mA / 12–14 h

All plastic parts (polyethylene, polypropylene), that come into contact with reagent solutions, are resistant to most of the usual solvents, acids and bases.

## Use

The TLC-sprayer is an alternative spray system operating without FCH and using compressed air as propellant. Accumulator operation and inductive charging make it possible to spray reagent solutions automatically anywhere. Unlike hydropneumatically

operated spray systems there is no dependence on the availability of compressed air or nitrogen lines. This makes for great flexibility of operation. The operating costs are low, because the TLC spray unit cannot be overloaded and a long working life of the accumulators is given. To increase ease of operation Merck supplies a range of exchange spray heads that can also be used as reagent bottle caps. This makes rinsing unnecessary when reagents are changed, so that time and solvent are saved. A service-kit with spare parts is also available for the TLC-sprayer.

## Ordering information

Order No	Description	Contents
8541.0001	Spray heads for TLC-sprayer	6 pieces
8542.0001	Service-kit for TLC-sprayer	10 sealing rings 5 air adaptors 5 replacement filters



Functional diagram



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## Ready-to-use spray solutions

Merck supplies a range of ready-to-use spray reagents for TLC. These are solutions of the particular reagent in ethanol, methanol/2-propanol or 2-propanol (contents 100 ml). Optimum spraying of the reagent is obtained in conjunction with the TLC-sprayer.

## Ordering information

Order No.	Reagent	Solvent	Application
1269.0100	Aniline phthalate	methanol/2-propanol	anions of oxygenated halo acids, reducing sugars
1994.0100	Bromocresol green	2-propanol	organic acids
9219.0100	2',7'-Dichlorofluorescein	2-propanol	saturated and unsaturated lipids
3722.0100	4-(Dimethylamino)benzaldehyde	2-propanol	sugars, steroids, terpenes
480.0100	Molybdato-phosphoric acid	2-propanol	steroids, sterols, lipids, reducing compounds
6705.0100	Ninhydrin	2-propanol	amines, amino acids, peptides, aminoglycoside antibiotics
7602.0100	Rhodamine B	ethanol	lipophilic substances, lipids

## Staining and derivatization reagents

Staining and derivatization reagents are also supplied for preparing your own spray solutions.

## Ordering information

Order No.	Reagent	Application
9676.0005	2',7'-Dichlorofluorescein	saturated and unsaturated lipids
9681.0025	Fluorescein	peroxides
9723.0001	Pinacryptol yellow	alkyl- and arylsulphonic acids
7546.0010	Quercetin	inorganic cations
59626.0001	Flavone reagent acc. to NEU (2-(diphenylboryloxy)-ethylamine	flavonoids, penicillic acid, anthocyanidines, carbohydrates, cinnamic acid derivatives

Reagent bottles with suitable screw caps are available for self-produced spray solutions.

## Ordering information

Order No.	Designation	Contents of
10647.0001	Reagent bottles 50 ml for TLC-sprayer	10 pieces
10646.0001	Reagent bottles 100 ml for TLC-sprayer	10 pieces



Should you have specific  
questions concerning  
thin-layer chromatography,  
please contact us:  
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- or contact our local representative.



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