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Analytical Products

TLC Tips & Tricks

Avoid Errors and Simplify Your Work with Our Practical TLC Tips

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AGENDA

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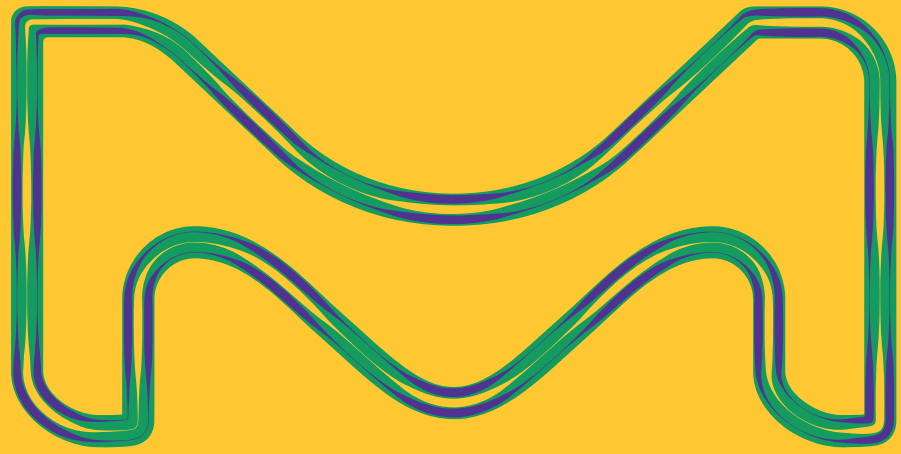
1. MERCK TLC overview

2.Thin-Layer Chromatography Process

3. Discover TLC tips and tricks

- Choice of Solvent System (Mobile Phase)
- Choice of TLC Layer (Stationary Phase)
- Pre-Conditioning TLC Plates
- Correct sample application
- Drying TLC Plates
- How to Saturate TLC Chambers
- Spraying TLC Plates for Derivatization
- Quantitative Evaluation with TLC Scanners



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MERCK TLC Overview

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TLC Plates to Fit Your Needs

As the leading supplier of thin-layer chromatography consumables, we offer

- ❑ An extensive portfolio of plates,
- ❑ Reagents and accessories for TLC,
- ❑ Preparative TLC,
- ❑ High performance TLC (HPTLC).
- ❑ Special MS-grade TLC and HPTLC plates for the perfect combination with mass spectrometry

Plate Backing or Support

Support/Backing	Advantages	Disadvantages
Glass	Rigid	Fragile
	Transparent	Cannot be easily cut into desired size
	Economical (reusable)	Heavy – high transport costs
	High chemical resistance	Thick (>1.0 mm) – about 5x more shelf space than aluminum or plastic-backed plates
	Most commonly used support	
	Good heat stability for charring	Backing highly susceptible to breakage (potential safety issue)
Aluminum Foil	Easy to handle/safe – resistant to breakage	Backing is not reusable
	Can be easily cut to desired dimensions with scissors	Not as chemically resistant as glass to reagents such as mineral acids and concentrated ammonia
	Thin (~0.15 mm) – minimal storage space	
	Lightweight – lower shipping costs	
	Solvent resistance	
	Strong adsorbent layer adherence – good for use with eluents containing high concentrations of water	
	Good heat stability	
	Can be stored in lab notebook	
Plastic (Polyester – PET)	Easy to handle/safe – resistant to breakage	Backing is not reusable
	Can be easily cut to desired dimensions with scissors	Lower heat stability – charring must be done at lower temperatures than with glass
	Thin (~0.2 mm) – minimal storage space	
	Lightweight – lower shipping costs	Flexible – adsorbent layer may be more susceptible to cracking
	Solvent resistance	
	Can be stored in lab notebook	

Adsorbent / Layers

Silica gel (unmodified, modified/bonded, chiral and high purity) is the most common TLC sorbent.

Aluminum oxide which exhibits similar selectivity, although slightly different, to silica is the second most common TLC sorbent.

Cellulose – available as either microcrystalline or fibrous cellulose.

Kieselguhr is a natural diatomaceous earth that can be used for the separation of polar or moderately polar compounds.

Binder

Our unique binder technology ensures a uniform and hard surface of the TLC plate that will not crack or blister

Polymeric (organic) binder:

- Traditional silica plates contain a polymeric (organic) binder of high molecular weight acrylic acid polymers for the most rugged plates, making sample handling and application easier.
- They also permit the use of higher water content in the developing solvent.

Gypsum:

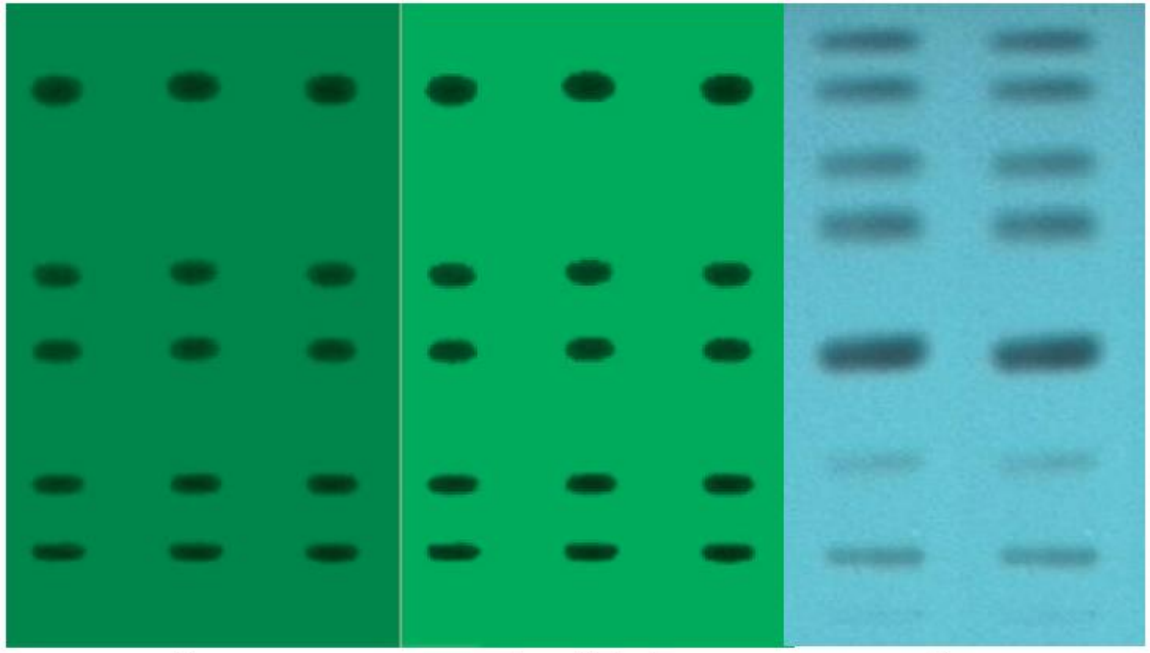
- Gypsum as a binder is recommended for TLC users in QA/QC labs following older Ph. Eur. monograph methods
- Our **TLC silica gel 60 G plates** are best suited for this approach.

Indicators

We also provide two kinds of inorganic fluorescent indicators for UV detection of colorless substances:

- ❑ The **green fluorescing F254**
- ❑ The **blue fluorescing, acid-stable F254s**
- ❑ Both of which fluoresce in UV light at an excitation wavelength of 254 nm.

- ❑ For superior identification of separated substances, our exceptional high-fluorescent **LuxPlates®** contain a higher amount of fluorescent indicator



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Thin-Layer Chromatography Process

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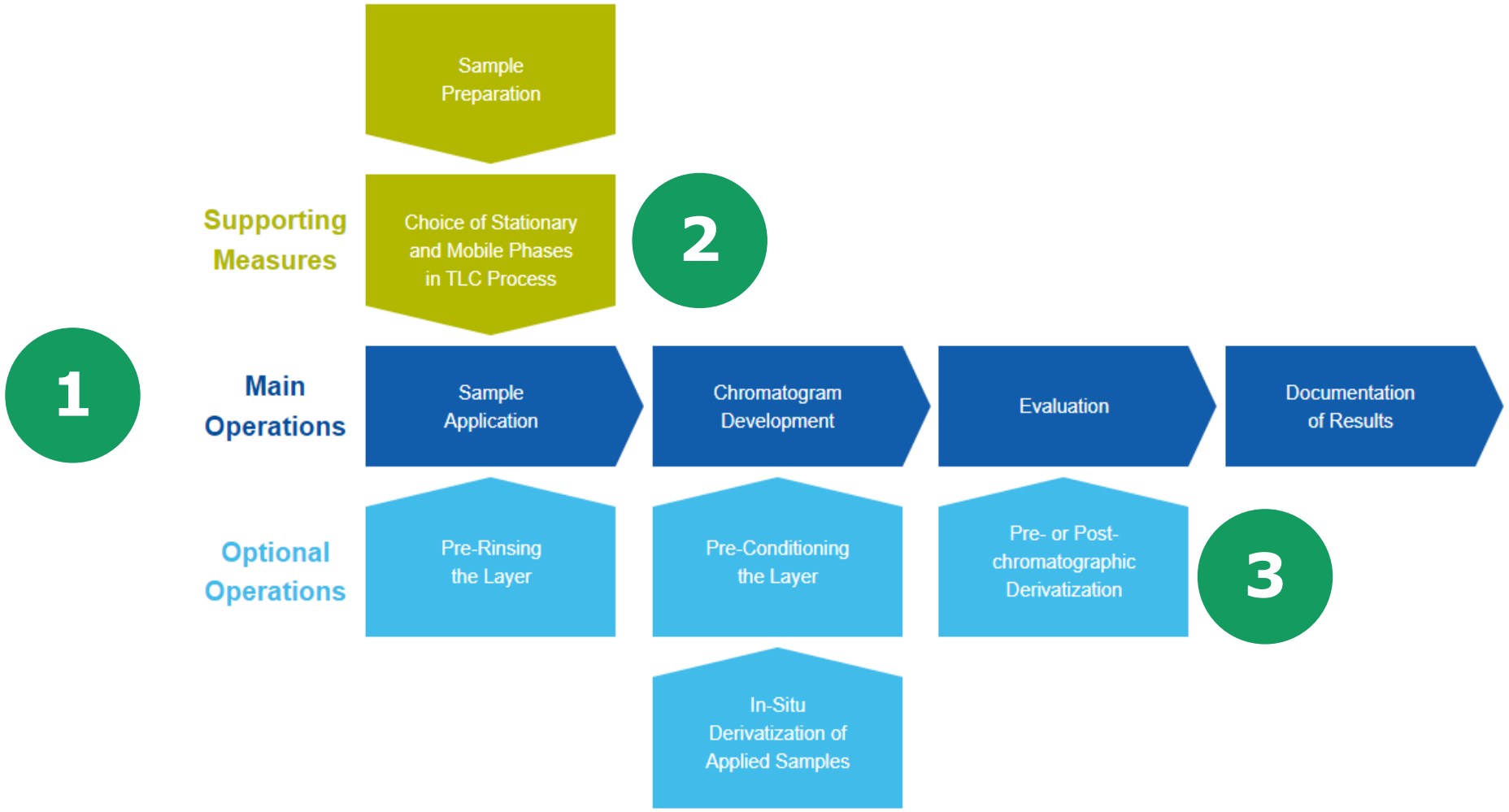
The TLC Working Principle

What causes a dissolved mixture to separate when applied to a TLC plate?

- The thin-layer chromatography process relies on capillary forces.
- During development of the chromatogram, the mixture of substances is first transported by the mobile phase, then resides on the stationary phase for a while, and is carried along again.
- The process repeats many times, and each substance is slowed down at different rates relative to the velocity of the mobile phase.
- The more a substance preferentially resides on the stationary phase, the slower its progress will be.
- Even substances with similar affinities for the two phases demonstrate differences in their chromatographic run, and can be separated.

Explore the TLC Process

The diagram below illustrates the complete thin-layer chromatography process



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Discover TLC tips and tricks

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TLC Tip 1: Choice of Solvent System (Mobile Phase)

The choice of solvent system is critical in thin-layer chromatography

- To choose the right solvent, start with **pure solvents of medium elution strength.**
- Perform spot tests to compare different solvent systems.
- Single solvents are seldom used in TLC; most solvent systems **contain several components, but keep it as simple as possible.**
- The solvent system must be capable of wetting the TLC layer.
- Use appropriate solvent purity
- Refer to scientific literature or pharmacopoeia monographs to facilitate your search.

The solvents are listed in increasing order of elution strength

			Velocity coefficient, k (mm²/s)
Lower elution strength	1	n-Heptane	11.4
	2	n-Hexane	14.6
	3	n-Pentane	13.9
	4	Cyclohexane	6.7
	5	Toluene	11.0
	6	Chloroform	11.6
	7	Dichloromethane	13.2
	8	Diisopropyl ether	13.2
	9	tert-Butanol	1.1
	10	Diethyl ether	15.3
	11	Isobutanol	1.6
	12	Acetonitrile	15.4
	13	Isobutyl methyl ketone	9.1
	14	2-Propanol	2.5
	15	Ethyl acetate	12.1
	16	1-Propanol	2.9
	17	Ethyl methyl ketone	13.9
Higher elution strength	18	Acetone	16.2
	19	Ethanol	4.2
	20	1,4-Dioxane	6.5
	21	Tetrahydrofuran	12.6
	22	Methanol	7.1
	23	Pyridine	8.0
	Sorbent		**TLC plate silica gel 60 F254 Merck**
	Type of chamber		**N-chamber with chamber saturation**
	Room temperature		**22 °C**
	Migration distance of solvent		**100 mm**

Source: Applied Thin-Layer Chromatography, Elke Hahn-Deinstrop, page 71

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TLC Tip 2: Choice of TLC Layer (Stationary Phase)

To help you select the optimal stationary phase for your analysis, the table below shows the most popular pre-coated TLC layers available and their typical applications.

Sorbent material	Chromatographic principle	Typical applications
Aluminum oxide	Adsorption chromatography due to polar interactions	Alkaloids, steroids, terpenes, aliphatic, aromatic and basic compounds
Cellulose	Depending on acetyl content transition from normal phase to reversed phase chromatography	Anthraquinones, antioxidants, polycyclic aromatics, carboxylic acids, nitrophenols, sweeteners
Kieselguhr	Commonly impregnated for reversed phase separations	Aflatoxins, herbicides, tetracyclin

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TLC Tip 2: Choice of TLC Layer (Stationary Phase)

Sorbent material	Chromatographic principle	Typical applications
Standard silica gel	Normal phase chromatography	Most frequent application of all TLC layers, Aflatoxins
Cyano-modified layer CN	Normal phase and reversed phase chromatography	Pesticides, phenols, preservatives, steroids
DIOL-modified layer		Steroids, hormones
Amino-modified layer NH_2	Anion exchange, normal phase and reversed phase chromatography	Nucleotides, pesticides, phenols, purine derivates, steroids, vitamins, sulfonic acids, carboxylic acids, xanthines

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TLC Tip 2: Choice of TLC Layer (Stationary Phase)

Sorbent material	Chromatographic principle	Typical applications
RP-2, RP-8, RP-18		Nonpolar substances (lipids, aromatics)
Silica gel 60 silanized		Polar substances (basic and acidic pharmaceutical active ingredients)
RP-18 W/UV254, wettable	Normal phase and reversed phase chromatography	Aminophenols, barbiturates, preservatives, nucleobases, PAH, steroids, tetracyclines, phthalates

Sorbent material	Chromatographic principle	Typical applications
LiChrospher® Si 60	Normal phase chromatography	Pesticides, phytopharmaceuticals

TLC Tip 3: Pre-Conditioning TLC Plates

Pre-conditioning TLC layers **protects them from humidity**, which could otherwise diminish their activity and affect chromatogram results.

- A common pre-conditioning method is **to place the TLC plate in a development chamber containing highly saturated salt solution** with a large amount of undissolved salt, and allowing the plate to condition for several hours. For reproducible results, make sure the solution contains sufficient undissolved salt!
- Other pre-conditioning methods include modifying the TLC layer by exposure to gas, or conditioning the plate with organic solvents, acids or bases.
- During sample application, cover the application area with a clean glass plate to maintain the layer's activity until development is completed.

TLC Tip 4: Correct sample application

The correct sample application on TLC plates is essential for accurate and reproducible separations. Below are a few ways you can avoid errors.

- ❑ Record the position of each sample on the data sheet.
- ❑ Cross out used lanes to prevent repeated application on any lane, and to ensure that no samples are omitted.
- ❑ Avoid applying samples too close to the plate's edge or to the solvent surface.
- ❑ Leave sufficient space between application areas.
- ❑ Ensure a consistent distance from the bottom edge of the plate for all samples.

TLC Tip 5: Drying TLC Plates

Highly volatile compounds (e.g. α-pinene)
Dry plates in a cool room to avoid sample evaporation prior to development.

Volatile compounds (e.g. essential oils applied with toluene or n-hexane)
Dry plates horizontally for a few minutes at room temperature before placing them in the development chamber.

Thermally stable substances (up to 1000 µg/lane from chloroform or methanol)
Apply uniform heat at a temperature close to the solvent's boiling point for around 20 minutes.

Thermally labile or oxidation-prone samples
Carry out several drying tests prior to separation.

Important: Keep exposure of plates to blowers as short as possible to protect the layer from airborne dirt particles.

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TLC Tip 6: How to Saturate TLC Chambers

TLC development can be performed in saturated or unsaturated chambers. **Chromatography in unsaturated chambers results in evaporation of the solvent from the layer, particularly near the front. This leads to higher solvent consumption, and higher Rf values.**

Chamber saturation method

- ❑ Line the chamber with strips of filter paper, leaving a gap for observation.
- ❑ Fill the chamber with solvent to a height of 0.5 to 1 cm.
- ❑ Carefully tilt the chamber to moisten the filter paper and equilibrate the chamber with solvent vapors. After a few minutes, the chamber is saturated with vapors.
- ❑ Place the TLC plate in the chamber carefully so that the solvent does not spill over the starting line. Contact between the side of the plate and the filter paper must also be avoided.
- ❑ Development can now proceed.

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TLC Tip 7: Spraying TLC Plates for Derivatization

Safety

- ❑ Airborne solvents may be toxic. Wear goggles, gloves and a dust mask while spraying, and ensure good ventilation.
- ❑ Avoid chlorinated hydrocarbons (CHC's) to protect yourself and the environment.

Challenges

- ❑ Spraying produces a less uniform coating than dipping or in-situ derivatization.
- ❑ Difficult to control reagent quantity while spraying.

Recommendations

- ❑ Always use fresh reagents for each application.
- ❑ Reagents stored for long periods should be thoroughly tested prior to usage.

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TLC Tip 8: Quantitative Evaluation with TLC Scanners

- ❑ Ensure that all chromatograph lanes are complete before placing the plate in the TLC scanner.
- ❑ For accurate analysis of complex sample mixtures, apply the sample as a band (instead of a spot).
- ❑ To establish the detection limit, use a blank lane outside the sample lanes for comparison.
- ❑ To avoid difficulties with linearity, keep the sample concentration range at a moderate limit.

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