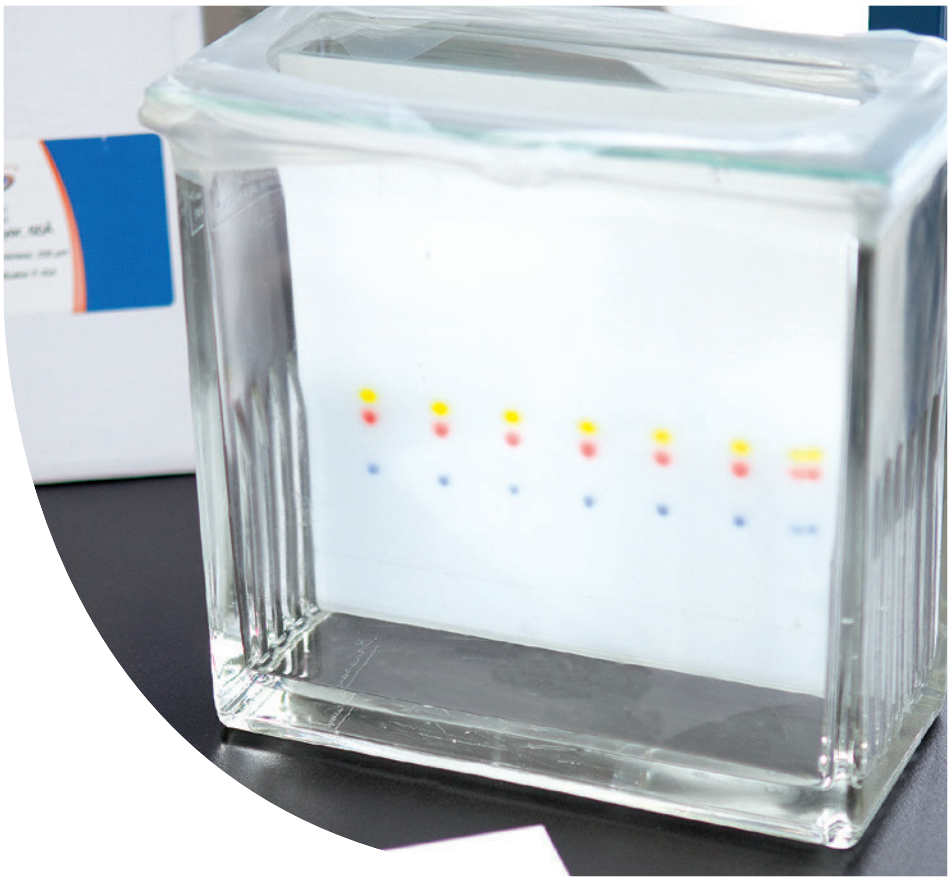
SiliaPlate™
TLC Plates



Distributed by

Greyhound Chromatography and Allied Chemicals
6 Kelvin Park, Birkenhead, Merseyside CH41 1LT United Kingdom
Tel: +44 (0)151 649 4000 Fax: +44 (0)151 649 4001
sales@greyhoundchrom.com

www.greyhoundchrom.com

Thin Layer Chromatography (TLC)

SiliCycle is your partner of choice for your purification and chromatography needs.

- Optimize your separation conditions by using the same silica gel as in your flash columns and cartridges.
- Made with an extra hard layer that ensures the plates don't loose silica on rubbing and heating.
- The consistent thickness of our Silia*Plate*™ ensures lot-to-lot reproducibility.

Introduction to Thin Layer Chromatography (TLC)

Thin layer chromatography (*TLC*) is a quick, simple and inexpensive analytical technique frequently used in various laboratories. It is used for reaction monitoring, compound purity evaluation as well as a rapid and cost-efficient selection and optimization of chromatographic conditions prior to purification by flash chromatography or HPLC.

Besides speed and low cost, TLC analysis presents other non-negligible advantages like the small quantity of compound required and high sample throughput capability (*up to 20 samples simultaneously*).

Silia*Plate* Features and Benefits

For over 18 years, SiliCycle has been offering a wide selection of TLC plates in various sizes (*plate size, thickness*) and chemistries (*10% Silver Nitrate, CN, C18, NH_2*). Silia*Plate* represents an efficient and economical alternative to other TLC plate manufacturers while demonstrating high separation power, which is due to the narrow particle size distribution silica gel used for manufacturing.

The extraordinary silica layer hardness combined to a homogeneous coating and layer thickness allow excellent separation. Each TLC batch is chemically and physically controlled by our Quality Control department to ensure lot-to-lot and layer-to-layer reproducibility.

« Many products have been successfully purified with the silica gel. We have had problems with other companies' TLC plates not running the same as their silica gel, but everything was fixed when we switched over to all SiliCycle products.. »

William Nguyen from Stanford University, Stanford, CA, USA

Types of plates available (*TLC/HPTLC/PLC*)

SiliCycle offers different types of plates for thin layer chromatography applications: classical TLC, high performance TLC (*also called HPTLC*) and preparative TLC (*PLC*). The plate types are selected based on the type of analysis required and the available budget.

Differences between classical TLC, HPTLC and PLC

Properties	Classical TLC	HPTLC	Preparative PLC
Application	Quick, inexpensive, flexible and portable separations	Highly sophisticated separation problems, complex samples	Purification on a TLC plate
Analysis	Qualitative	Qualitative & Quantitative	Quantitative
Detection	UV - Stains	Instrumented analysis (*use of scanners for detection*)	UV
Price	Lower prices than HPTLC	Higher prices than TLC	-
Distribution [Mean Particle Size]	5 - 20 µm [10 - 12 µm]	4 - 8 µm [5 - 6 µm]	5 - 40 µm [25 µm]
Layer Thickness	250 µm	200 µm	0.5 mm, 1 mm, 2 mm
Typical Sample Volume	1 – 5 µL	0.1 – 0.5 µL	5 – 20 µL

TLC Backings

TLC plates are available with different backings (*also called supports*): rigid (*glass-backed*) or flexible sheets (*aluminum & plastic-backed*). Glass backed plates are the most frequently used due to the ease of handling, transparency (*spot can be seen on both sides*) as well as the chemical resistance and inertness of the support. However, glass plates also present certain disadvantages like superior fragility and higher weight over flexible backings. On the other hand, aluminum and plastic backings also offer both pros and cons as presented in the table below.

TLC Backings Comparison

Properties	Glass	Aluminum	Plastic
Approximate Thickness	1.5 mm	1.5 mm	2 mm
Total Weight	High	Low	Medium
Heating Stability	High	High	Below 175°C
Fragility	High	Low	Low
Scissors Cut	Impossible	Easily	Possible
Chemical Resistance Against			
Mineral Acids	High	Low	High
Bases (*ammoniac*)	High	Low	High

Available Sorbents

Various adsorbents can be used for TLC coating; silica, alumina, florisil, etc. However, silica gel is probably the most versatile since it covers almost all types of separation (*if the right solvent system is selected*). More than 80% of all purifications are performed using silica gel as the adsorbent.

The particle size distribution used for the silica is related to the nature of the plate. For standard TLC, silica gel with a mean particle size of 10 - 14 µm is used compared to HPTLC where a smaller particle size is required. In both cases, pore diameter is always 60 Å. Some functionalized silica gels like reversed-phase (*C18, C8, Amine, Cyano, Diol,* ...) and specialty (*Silver Nitrate*) plates can also be used as TLC adsorbent for particular needs.

The two most popular modes of separation employed in TLC are normal and reversed phases. In normal phase separation, the mobile phase is less polar than the stationary phase. Inversely, in reversed mode, the mobile phase (*usually a mixture of water and organic solvent*) is more polar than the stationary phase (*C18*).

Layer Thickness

The layer thickness is related to the nature of the analysis (*analytical or preparative*) as well as the performance of the plate (*TLC or HPLTC*). The most common layer thicknesses are 150 µm (*HPTLC plates*), 200 – 250 µm (*analytical TLC plates*) and 500 - 2,000 µm (*preparative TLC plates*).

Binder & UV Indicator

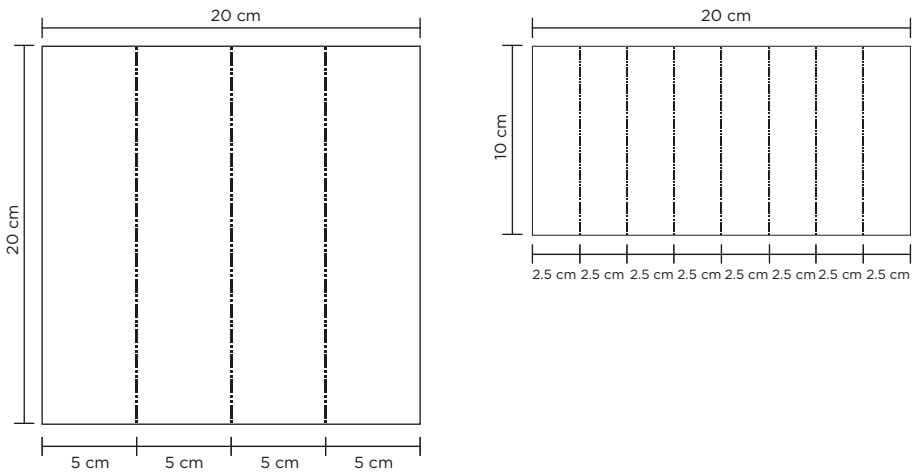
All standard Silia*Plate* products are made with a Gypsum binder and have an UV indicator (*F254*). Contact us for custom products.

Plate Size

Silia*Plate* TLC plates are available in the following standard sizes depending on the coating used: 20 x 20 cm, 10 x 20 cm, 5 x 20 cm, 5 x 10 cm & 10 x 10 cm. Also for your convenience, SiliCycle provides ready to use micro TLC plates in 2.5 x 10 cm, 2.5 x 7.5 cm & 2.5 x 5 cm formats.

An interesting compromise between standard and micro plate sizes is our Scored Silia*Plate* (*glass backing*). Two different formats are available and possible cut combinations are shown in the image below.

- 20 x 20 cm plates scored to get four 5 x 20 cm plates (*or multiple of 5 cm width*).
- 10 x 20 cm plates scored to get seven 2.5 x 10 cm plates (*or multiple of 2.5 cm width*).



SiliaPlate TLC Plates Portfolio

SiliCycle offers the possibility to analyze reactions on thin layer chromatography support and rapidly develop optimized purification conditions for efficient transfer to flash columns packed with the same SiliaFlash silica support. Maximize the benefits by using our *UltraPure* SiliaPlate TLC plates with an extra hard layer of silica. For your convenience, SiliCycle offers different sizes, choice of backings, reversed-phase & specialty plates. Contact us for more information.

Various attribute combinations are possible with SiliaPlate TLC plates and are summarized in the table below.

SiliaPlate TLC Plates Variety of Attributes

Properties	TLC		HPTLC NEW
	Analytical	Preparative	
Backing Available			
Glass	Yes	Yes	Yes
Aluminum	Yes	No	No
Plastic	Yes	No	No
Adsorbent Available			
Bare silica	Yes	Yes	Yes
Silica - functionalized	No	Yes	Yes
Silica Specifications			
Mean Particle Size	10 - 14 µm	20 - 25 µm	≤ 10 µm
Mean Pore Diameter	60 Å	60 Å	60 Å
Type of Plate Available			
Scored Plate Available	Yes	Yes	No
Channeled Plate Available	Yes	No	No
Layer Thickness	Glass: 250 µm Flexible: 200 µm	Glass: 500 µm, 1,000 µm Flexible: 1,500 µm, 2,000 µm	Glass: 150 µm
Typical Possible Plate Size*	2.5 x 5 cm; 2.5 x 7.5 cm; 2.5 x 10 cm; 5 x 10 cm; 5 x 20 cm; 10 x 20; 20 x 20 cm	20 x 20 cm	2.5 x 5 cm; 2.5 x 7.5 cm; 2.5 x 10 cm; 5 x 10 cm; 5 x 20 cm; 10 x 20; 20 x 20 cm

*For the glass-backing TLC plates.

« We had tried working with TLC plates of another brand and realized that the SiliCycle brand was the most durable and long-lasting as well as clear when visualizing with UV light so we switched back. »

Jessica Kisunzu from UC Berkeley, Berkeley, CA, USA

Silia*Plate* Ordering Information.

Silia*Plate* TLC with Glass Backing				
SiliCycle PN	Product Name	Plate Size (cm)	Thickness (µm)	#/box
Analytical Silia*Plate* Glass				
TLG-R10011B-423	Micro Silia*Plate* Glass	2.5 x 5	250	25
TLG-R10011B-124	Micro Silia*Plate* Glass	2.5 x 7.5	250	100
TLG-R10011B-2575B NEW	Micro Silia*Plate* Glass (*bulk*)	2.5 x 7.5	250	384
TLG-R10011B-624	Micro Silia*Plate* Glass	2.5 x 10	250	100
TLG-R10011B-527	Silia*Plate* Glass	5 x 10	250	200
TLG-R10011B-424	Silia*Plate* Glass	5 x 20	250	100
TLG-R10011B-723	Silia*Plate* Glass	10 x 20	250	25
TLG-R10011B-2020 NEW	Silia*Plate* Glass	20 x 20	250	20
TLG-R10011B-323	Silia*Plate* Glass	20 x 20	250	25
Scored Analytical Silia*Plate* Glass				
TLGSR10011B-723	Silia*Plate* Glass (*scored*)	10 x 20	250	25
TLGSR10011B-423	Silia*Plate* Glass (*scored*)	20 x 20	250	25
Channeled Analytical Silia*Plate* Glass (*with Preadsorbent Zone*)				
TLGCZ-R10011B-323 NEW	Channeled Silia*Plate* Glass	20 x 20	250	25
Preparative Silia*Plate* Prep (*Glass Preparative*)				
TLG-R10011B-333	Silia*Plate* Prep	20 x 20	500	25
TLG-R10011B-341	Silia*Plate* Prep	20 x 20	1,000	25
TLG-R10011B-353	Silia*Plate* Prep	20 x 20	2,000	25
Scored Silia*Plate* Prep (*Glass Preparative*)				
TLGSR10011B-333 NEW	Silia*Plate* Prep Glass (*scored*)	20 x 20	500	25
TLGSR10011B-341 NEW	Silia*Plate* Prep Glass (*scored*)	20 x 20	1,000	25
TLGSR10011B-350 NEW	Silia*Plate* Prep Glass (*scored*)	20 x 20	2,000	25
Silia*Plate* Prep C18 (*Glass Preparative*)				
TLG-R30411B-341	Silia*Plate* C18 Prep Glass	20 x 20	1,000	25

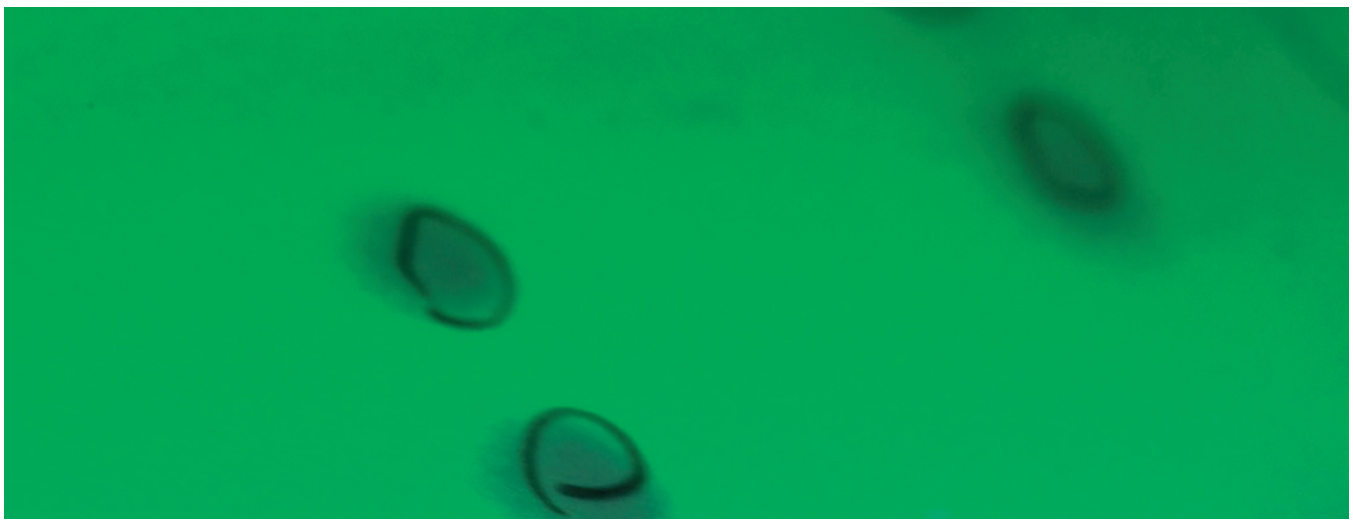
Silia*Plate* TLC with Flexible Backings				
SiliCycle PN	Product Name	Plate Size (cm)	Thickness (µm)	#/box
Silia*Plate* Al (*Aluminum*)				
TLA-R10011B-2575 NEW	Micro Silia*Plate* Aluminum	2.5 x 7.5	200	200
TLA-R10011B-323	Silia*Plate* Aluminum	20 x 20	200	25
Silia*Plate* Al C18 (*Aluminum*)				
TLA-R30411B-303	Silia*Plate* Aluminum C18	20 x 20	200	25
Silia*Plate* Pl (*Plastic*)				
TLP-R31001B-2575 NEW	Micro Silia*Plate* Plastic	2.5 x 7.5	200	200
TLP-R31001B-323	Silia*Plate* Plastic	20 x 20	200	25

NEW

SiliaPlate HPTLC Silica with Glass Backing (*Thickness: 150 microns, 25 plates/box*)			
SiliCycle PN	Plate Size (cm)	SiliCycle PN	Plate Size (cm)
SiliaPlate Silica HPTLC			
HPTLG-R10011B-1010	10 x 10	HPTLG-R10011B-2020	20 x 20
Scored SiliaPlate Silica HPTLC			
HPTLGSR10011B-1010	10 x 10 (to 5 x 5 cm)	HPTLGSR10011B-1020	10 x 20

Functionalized SiliaPlate HPTLC (*Thickness: 150 microns, 25 plates/box*)			
SiliCycle PN	Plate Size (cm)	SiliCycle PN	Plate Size (cm)
SiliaPlate C18 HPTLC			
TLG-R30411B-213	10 x 10	TLG-R30411B-303	20 x 20
SiliaPlate C8 HPTLC			
TLG-R31011B-203	10 x 10	TLG-R31011B-303	20 x 20
SiliaPlate C2 HPTLC			
TLG-R32611B-203	10 x 10	TLG-R32611B-303	20 x 20
SiliaPlate NH_2 (*Amine*) HPTLC			
TLG-R52011B-203	10 x 10	TLG-R52011B-303	20 x 20
SiliaPlate CN (*Cyano*) HPTLC			
TLG-R38011B-203	10 x 10	TLG-R38011B-303	20 x 20
SiliaPlate Diol HPTLC			
TLG-R35011B-203	10 x 10	TLG-R35011B-303	20 x 20
SiliaPlate Ag (*Silver Nitrate 10% impregnated*) HPTLC			
TLG-R23511B-423	5 x 20	TLG-R23511B-303	20 x 20

Trial Package of Functionalized SiliaPlate HPTLC: TLG-R1234511B-723
[5 plates (10 x 20 cm) of each SiliaPlate C18, C8, C2, NH_2 & CN scored to 2.5 x 10 cm]



Silia*Plate* TLC Accessories

Silia*Plate* TLC Developing Chamber

The most commonly used accessories to develop a TLC plate.

AUT-0160 Silia*Plate* Cylinder Micro TLC Developing Chamber (*1/box*)

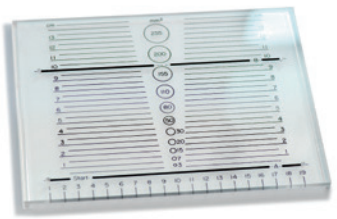
AUT-0161 Silia*Plate* Rectangular TLC Developing Chamber (*1/box*)

Other Silia*Plate* TLC Accessories

AUT-0162 Silia*Plate* TLC Cutter

AUT-0163 Silia*Plate* TLC Spotting Capillary Tubes

AUT-0164 Silia*Plate* TLC Spotting Guide



An Ideal Partnership Between SiliCycle and Society AR2i

SiliCycle has entered into an exclusive world strategic specialty distribution partnership with the Society AR2i specialized in the conception and the manufacturing of innovative devices in the field of Thin-Layer Chromatography since 1994.

Chromimage® Documentation

- Perform qualitative analysis on TLC plates in a few minutes.
- Detect and numerize your TLC plates under UV254 nm and visible mode.
- Classify and archive your TLC analysis under several storage formats (*jpg, eps, pdf, etc.*).
- Suitable for reading 10 x 10 cm, 10 x 20 cm and 20 x 20 cm plates.

PN: AUT-0165

Derivapress® System

It's simple as opening and closing a book: the Derivapress immersion derivatization system provides a cost-effective, efficient and safe alternative to perfect this essential stage of TLC and to move towards densitometric measurements like quantitative and semi-quantitative TLC.

Furthermore Derivapress complies with the GLP requirements and can be used in 21 CFR Part 11 work environments.

PN: AUT-0166

Thin Layer Chromatography Practical Guide

Select a Stationary Phase

As almost 80% of all separations can be performed using silica gel plates, it is suggested to try using this coating. However, for acid sensitive compounds, alumina is probably a better choice (*useful for amine purification*). If you are working with highly polar compounds, reversed-phase mode is more suitable.

Select a Mobile Phase (*Solvent Systems*)

The selection of the mobile phase (*also called solvent system or eluent*) is perhaps the most important parameter to achieve efficient thin layer chromatography separation. It is based on the compound's solubility with the solvent and the difference in the affinity for the mobile phase versus the stationary adsorbent (*silica*).

In normal phase chromatography, where non-polar solvents such as hexane or pentane are used, non-polar compounds will move up the plate while most polar compounds will stay on the baseline. Inversely, polar solvents will allow polar compounds to move off the origin. The most suitable solvent system is the one that moves all components off the baseline with Rf values between 0.15 and 0.85 (*ideally, close to 0.2 - 0.4*).

Remember that it is not always possible in TLC but should be possible in flash chromatography where solvent gradients can be used.

For most applications, a common solvent system to start with is 1:1 Ethylacetate (*EtOAc*) / Hexane. Varying the ratio can have a pronounced effect on the Rf. If it is not working, then try: Methanol (*MeOH*) / Dichloromethane (*DCM*) (*1:99 – 10:90*); or toluene with acetone, EtOAc, or DCM.

Remember: To increase the compound's Rf, increase the polarity of the mobile phase; increase the ratio of the polar solvent or choose another solvent. Inversely, to decrease Rf, decrease the polarity of the eluent.

Rules of Thumb

- **Standard compounds (*most popular solvent system*)**: 10 - 50% EtOAc/Hexane
- **Polar compounds**: 100% EtOAc or 5 - 10% MeOH/DCM
- **Non-polar compounds**: 5% EtOAc (*or ether*) / Hexane or 100% Hexane
- **For basic compounds**: (*amine or nitrogen containing*), it could be useful or required to add a small quantity of triethylamine (Et_3N) to the solvent mixture (*0.1 – 2.0% but typical quantity is 0.1%*) or 1 - 10% ammonia (NH_3) in MeOH/DCM.
- **For acidic compounds**: it could be useful to add acetic (*AcOH*) or formic acid (*FA*) to the solvent mixture (*0.1 - 2.0%*).

Reversed-phase mode

In reversed-phase chromatography, the typical solvent systems are:

- Mixtures of water or aqueous buffers and water miscible organic solvents such as acetonitrile *(ACN),* methanol, and tetrahydrofuran *(THF*). Other solvents can be used such as ethanol (*EtOH*) & isopropanol (*IPA*).
- If needed, to improve peak shape in flash chromatography, 0.1% of acetic, formic or trifluoroacetic acid (*TFA*) can be added to the solvent system.

« Have given your products to other folks within organisation and used it myself with great success (both the Prep SPE, HPLC columns, TLC plates, and silica gel).. »

Kerry M. Keertikar, Merck Research Labs, Kenilworth, NJ, USA

TLC Plate Preparation

Using a pencil, lightly draw a straight-line parallel to the width of the plate at about 1 cm from the base end of the plate. Sample application will be done on this line called baseline or origin.

Note: Never use a pen because ink can move with some solvents used as eluent.

Sample preparation

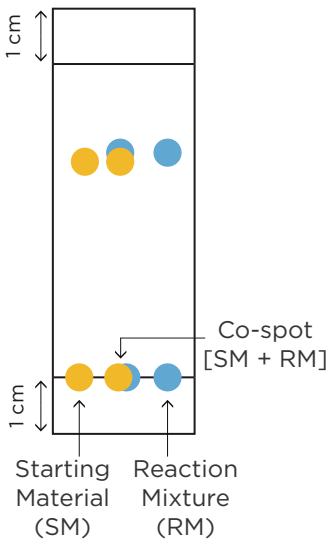
Thorough sample preparation is a prerequisite for an optimal and efficient TLC separation. Typical sample preparation processes could consist in a sample crushing, filtration, extraction or concentration of the product of interest.

Sample Application

Sample preparation will differ depending on the nature of the plate (*analytical or preparative*). For analytical plates, because thin layer chromatography is extremely sensitive, it is really important to apply a small quantity using a glass capillary (*or a micro pipette*) to get optimal resolution. For preparative plates, apply a series of small adjacent spots to form a band or a streak using a glass capillary (*or a microliter syringe*). In both cases, a spotting guide can be used to facilitate sample application.

Co-spotting

For analytical chromatography, co-spotting is frequently used for similar polarity products. This consists to apply on the same spot, the starting material and reaction mixture as shown by the image below.



TLC Plate Development

The most commonly used method to perform thin layer chromatography separation is to place vertically the TLC plate inside a sealed developing chamber to ensure solvent saturation. Place approximately 0.5 cm of the suitable solvent system inside the chamber. Slowly place the TLC inside the chamber and allow the eluent to travel up the plate until it gets to 1 cm from the top of the plate. Immediately remove the plate and draw a line along the solvent front.

Note: for optimal solvent saturation, a filter paper can be added inside the TLC chamber. This also prevents eluent evaporation. The solvent level needs to be below the baseline; otherwise the spots will be dissolved.

TLC Plate Visualization

If components of the reaction are colored, no visualization method is required (*spots can be seen directly on the silica layer*). However, most of the time it is not the case, therefore one of the methods described below should be used to reveal the spots.

Non-destructive methods

As a general visualization procedure, before treating the TLC plate with any destructive methods, UV-active compounds can be viewed under an ultraviolet lamp (*usually for polyconjugated compounds like benzophenones and anthracenes*). Furthermore, an iodine chamber can be useful for thiols, phosphines, and alkenes but it works in about 50% of cases for alkanes. It is recommended to circle the spots with a pencil on the TLC plate prior to visualization by destructive methods.

Destructive methods

For compounds that are not UV-active, there are several varieties of stains that can be used depending on the nature of the compound of interest. To use a stain, simply dip the TLC plate into the staining solution as quickly as possible, and then immediately absorb the excess stain with paper and heat carefully with a heat gun or on a hot plate at 110°C until spots are revealed. See next two pages.

Chromatogram Interpretation

Retention factor (*Rf*) definition

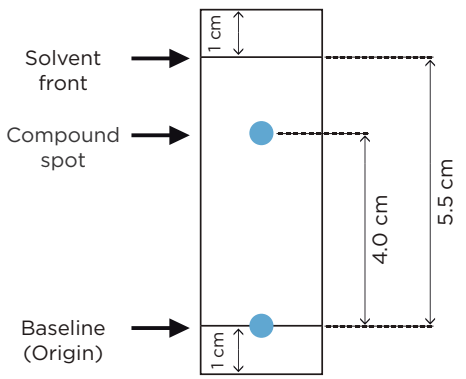
Retention factor analysis is used to evaluate if the solvent system is adequate. Rf is defined as the distance traveled by the compound divided by the distance traveled by the solvent front. This means: the larger the Rf value of a compound, the larger is the distance traveled by the compound. In other words, when comparing Rf values of various compounds under identical chromatography conditions, the compound with the larger Rf is less polar because it interacts less strongly with the polar adsorbent on the plate.

Remember, a good solvent system is one that moves all components off the baseline with Rf values between 0.15 and 0.85 (*ideal Rf is 0.2 - 0.4*). Otherwise, when possible, it is preferable to chose another solvent system.

$$\text{Retention factor (Rf)} = \frac{\text{distance traveled by the compound}}{\text{distance traveled by the solvent front}}$$

Rf calculation based on the example shown here:

Rf = 4.0 cm / 5.5 cm = 0.73



Prediction of Column Volumes (CV)

TLC data can be used to predict column elution based on the relationship between the retention factor and the column volume. CV is the number of column volumes required to elute the component from the column regardless of column dimensions [(*bed volume*) – (*volume of packing*)].

$CV = 1 / Rf$ & $\Delta CV = 1 / Rf_1 - 1 / Rf_2$

The greater the ΔCV, the greater will be the separation and resolution between the spots (easier separation). A bigger ΔCV will therefore allow more sample to be loaded onto the column.

Described below, are the most frequently used TLC visualization methods (also called stains) in alphabetical order.

Stains for Thin Layer Chromatography

Name	Visualization of...	Stain Recipe	Comments
***p*-Anisaldehyde #1**	**Universal Stain** *Good for nucleophiles and oxygenated compounds*	**Prepare stain as follows** • 2 mL of glacial acetic acid • 5 mL of *p*-anisaldehyde • 7 mL of conc. sulfuric acid • 185 mL 95% ethanol ***Tip**: Add dropwise the acid at the end and stir vigorously.*	**Visualization Colors** • Spots: Various colors • BG: Orange to pink **Appropriate Storage** • Aluminum wrapped at 0°C
N.B.: Tends to be insensitive to alkenes, alkynes and aromatic compounds unless other functional groups are present.			
***p*-Anisaldehyde #2**	**Acronycine** **Cineoles** **Terpenes**	**Prepare stain as follows [1:10:20:80]** • *p*-anisaldehyde • perchloric acid • acetone • water	**Visualization Colors** • Spots: Various colors • BG: Orange to pink **Appropriate Storage** • Aluminum wrapped at 0°C
Bromocresol Green	**Acidic groups (pK_a < 5)** **Carboxylic Acids**	**Prepare stain as follows** • 0.04 g of bromocresol green • 100 mL of 95% ethanol • 0.1 M solution of sodium hydroxide ***Tip**: Add the base slowly at the end until the solution turns pale blue.*	**Visualization Colors** • Spots: Yellow to green • BG: Blue **Appropriate Storage** • Aluminum wrapped at 0°C ***Heating NOT required***
Cerium Molybdate (CAM or Hanessian's Stain)	**Universal Stain** *Good for peptides*	**Prepare stain as follows** • 12 g of ammonium molybdate • 0.5 g of ceric ammonium molybdate • 15 mL of conc. sulfuric acid • 235 mL of water	**Visualization Colors** • Spots: Blue • BG: White **Appropriate Storage** • Aluminum wrapped
N.B.: Highly sensitive stain; very low concentration of product may appear as a significant impurity.			
Cerium Sulfate ($Ce(SO_4)_2$)	**Difficultly stainable compounds**	**Prepare stain as follows** • 15% aqueous sulphuric acid saturated with ceric sulfate	**Visualization Colors** • Spots: Black • BG: Yellow to white
Chromic Acid	**Difficultly stainable compounds**	**Prepare stain as follows** • 2.5 g of potassium chromate • 100 mL of 20% sulfuric acid in water	**Visualization Colors** • Spots: Orange to green • BG: Yellow to red
Cobalt Chloride ($CoCl_2$)	**Universal stain** *Used in conjunction with PMA when this one is not effective enough*	**Prepare stain as follows** • 2 g of cobalt chloride • 100 mL of water • 10 mL of conc. sulfuric acid ***Tip**: simply dip PMA treated plate in $CoCl_2$ solution.*	**Visualization Colors** • Spots: Various colors • BG: Pink ***Heating NOT required***
***p*-Dimethylamino-benzaldehyde** (PDAB or Ehrlich's Reagent)	**Amines** **Indoles**	**Prepare stain as follows** • 0.5 g of *p*-dimethylamin-benzaldehyde • 10 mL of conc. hydrochloric acid • 40 mL of acetone (or 95% ethanol)	**Visualization Colors** • Blue

Stains for Thin Layer Chromatography (*Con't*)

Name	Visualization of...	Stain Recipe	Comments
2,4-Dinitrophenyl-hydrazine (DNP)	**Aldehydes** **Ketones**	**Prepare stain as follows** • 12 g of 2,4-dinitrophenylhydrazine • 60 mL of conc. sulfuric acid • 80 mL of water • 200 mL of 95% ethanol	**Visualization Colors** • Spots: Yellow to red • BG: Light orange ***DO NOT HEAT dipped plate***
Dragendorff Reagent	**Nitrogenous Compounds** *Alkaloids, amines, organics bases, etc.* **Phenols**	**Prepare stain as follows** **Solution A** • 1.7 g of bismuth nitrate • 80 mL of water • 20 mL of acetic acid **Solution B** • 40 g of potassium iodide • 100 mL of water ***Tip**: mix 5 mL of each solution A and B to a solution of 20 mL of acetic acid in 70 mL of water.*	**Visualization Colors** • Spots: Orange to red • BG: Yellow **Appropriate Storage** • Aluminum wrapped **Stain Shelf-Life** • One or two weeks • Solutions A and B are long term storable ***DO NOT HEAT dipped plate***
Ferric Chloride ($FeCl_3$)	**Phenols**	**Prepare stain as follows** • 2 g of ferric chloride • 102 mL of 0.5N hydrochloric acid	**Visualization Colors** • Spots: Red • BG: Yellow
Iodine	**Unsaturated & Aromatic Compounds**	**Prepare stain as follows** • Iodine crystals in an amber bottle	**Visualization Colors** • Spots: Dark brown • BG: Light brown
N.B.: iodine stain can be removed by heating.			
Morin Hydrate (Hydroxy Flavone)	**Universal stain** *Fluorescently active*	**Prepare stain as follows** • 0.1% of morin hydrate in methanol ***Tip**: by weight.*	**Visualization Colors** • Spots: Various colors • BG: White
Ninhydrin (Indanetrione Hydrate)	**Amino Acids** **Amino Sugars** **Amines**	**Prepare stain as follows** • 1.5 g of ninhydrin • 3 mL acetic acid • 100 mL of *n*-butanol	**Visualization Colors** • Spots: Various colors • BG: White
Phosphomolybdic Acid (PMA)	**Universal stain** *Very effective against dilute sample*	**Prepare stain as follows** • 10% of PMA solution in ethanol • or 10 g of PMA in 100 mL ethanol	**Visualization Colors** • Spots: Dark green to black • BG: Light green
Potassium Permanganate ($KMnO_4$)	**Olefins** **Readily oxidized groups** *Alcohols, aldehydes, alkenes, alkynes, etc.*	**Prepare stain as follows** • 1.5 g of potassium permanganate • 10 g of potassium carbonate • 1.25 mL of 10% sodium hydroxyde • 200 mL of water	**Visualization Colors** • Spots: Yellow to light brown • BG: Purple to pink **Stain Shelf-Life** • Three months
N.B.: Can be used for detection of alcohols, amines, sulfides and mercaptans groups when gently heated.			
Vanillin	**Universal stain** *Very effective for same polarity products (Rf)*	**Prepare stain as follows** • 15 g of vanillin • 250 mL of 95% ethanol • 2.5 mL of conc. sulfuric acid	**Visualization Colors** • Spots: Various colors • BG: Light tan

(**N.B.**: Shaded lines refer to "universal stains". Occasionally, spots can be seen more clearly from glass side with glass backed TLC plate. Otherwise mentioned, stains are long-term stable when stored in a tightly-closed container to prevent solvent evaporation. "BG" stands for "background".)

Silia*Plate* TLC Troubleshooting

Problem: Streaking or elongated spot rather than a defined spot?

Possible Solutions:

- Sample was overloaded: run the TLC again using a more diluted solution of your sample.
- In presence of a base sensitive compound: try to add acetic or formic acid to the eluent (*0.1 - 2.0%*).
- In presence of an acid sensitive compound: try to add triethylamine to the eluent (*0.1 - 2.0%*) or 1 - 10% ammonia in MeOH/DCM. If it is not working use Alumina as TLC coating.
- In presence of too highly polar compounds: try using a specialized silica TLC plate like reversed-phase (*C18 for example*).

Problem: Unable to see any spots on the TLC?

Possible Solutions:

- If you have not been able to visualize any spots on your TLC using UV light, try another method; maybe your compound is not UV-active.
- Maybe your sample is too diluted. Try to apply several times your sample on the same spot (*do not forget to dry solvent between each application for optimal results*) or to concentrate your solution.
- Make sure the solvent level inside the tank is lower than the spotting line to avoid sample dissolution by the eluent.

Problem: How to monitor a reaction in presence of similar Rfs for both starting materials and product of interest?

Possible Solutions:

- Try the co-spotting method.
- Try to visualize the plate using anisaldehyde or molybdene. Spot color or brightness differ for two compounds when using these stains.
- If none of the two previous solutions work, change solvent systems (*use another class of solvent*).

Tips*: in chromatography, there are three classes of solvent systems providing significantly different results:*

1: Mixture of polar/hydrocarbon solvents (*i.e.: EtOAc/Hexane; Ether/Petroleum ether*).

2: Mixture of polar/dichloromethane solvents (*examples of polar solvent: Ether, EtOAc, MeOH*).

3: Mixture of polar/benzene (*or toluene*) solvents (*examples of polar solvent: Ether, EtOAc, MeOH*).

Problem: Compounds stay too close to the baseline or solvent front.

Possible Solutions:

- Too close to the baseline: your eluent is not polar enough; increase the proportion of polar solvent in the same solvent system or chose a more polar solvent.
- Too close to the solvent front: inversely, your eluent is too polar; decrease the proportion of polar solvent in the same solvent system or chose a less polar solvent.