

Silica-Gel Thin Layer Chromatography (TLC) Protocols

Running a TLC plate

Cut TLC plate, 5 cm tall by desired width

Dissolve compound in appropriate solvent (between 0.1 - 10 mg/mL)

Spot a small amount of compound 0.5 cm from bottom of TLC plate. The spot should have as small a diameter as possible. Let the solvent evaporate.

Make TLC solvent and place in chamber. Wait 1-5 min to equilibrate the atmosphere in TLC chamber.

Place TLC in chamber and let solvent front run up the plate. When the plate has run far enough (solvent front approx 1 - 0.5 cm from top of plate) remove plate from TLC chamber

Let TLC plate dry and then visualize

Equipment for TLC

TLC plates: Glass backed plates are best since you can look through the glass at the back side of the TLC plate. Sometimes the best information is found on this side. We always use EM Silica Gel 60 F₂₅₄ glass backed plates in the 20cm x 20cm format. We cut the plates into four 5cm x 20 cm strips using a glass cutter and then cut TLC plates from these strips as needed.

TLC solvent chamber: You can buy expensive chambers for running TLC, we don't. We use wide-mouth clear glass 125 mL jars (Fisher 02-911-775). Make sure you place into your TLC chamber a piece of filter paper that reaches from the bottom of the top. This will absorb solvent and help prevent evaporation of solvent from you TLC plate, making your TLCs more reproducible.

TLC spotters: We make our own from glass pipettes. We find them to be far superior to commercial spotters for a number of reasons. The internal diameter is much smaller in our home made spotters. This reduces carryover between samples when spotting a TLC plate, allowing you to use the spotter multiple times. Also, we often need very long TLC spotters to sample reactions under inert atmosphere. Making our own spotters allows us to adjust their length to suit our needs.

TLC solvents: Finding the right solvent is the key to getting good data. Here are some of our favorites.

| | | | | |
|---|---------------------|-----------------------|---------------------------------|---------------------|
| | common solvents | less common solvents | | |
| | → | | | |
| ↑ | non-polar solvents | | | |
| | | 5-10% Acetone/hexanes | 5-10% Et ₂ O/hexanes | |
| | 5-10% EtOAc/hexanes | 25% Acetone/hexanes | 25% Et ₂ O/hexanes | |
| | 25%EtOAc/hexanes | 50% Acetone/hexanes | 50% Et ₂ O/hexanes | 5-10% EtOAc/benzene |
| | 50%EtOAc/hexanes | 75% Acetone/hexanes | 75% Et ₂ O/hexanes | 25%EtOAc/benzene |
| | 75%EtOAc/hexanes | 100% Acetone/hexanes | 100% Et ₂ O/hexanes | 50%EtOAc/benzene |
| | 100%EtOAc/hexanes | | | 75%EtOAc/benzene |
| | | | | 100%EtOAc/benzene |
| | 2% MeOH/DCM | | | |
| | 5% MeOH/DCM | 2% MeOH/EtOAc | | |
| | 10% MeOH/DCM | 5% MeOH/EtOAc | 2% MeOH/Actone | |
| | 20% MeOH/DCM* | 10% MeOH/EtOAc | 5% MeOH/Acetone | |
| | | 20% MeOH/EtOAc* | 10% MeOH/Acetone | |
| | | | 20% MeOH/Acetone* | |
| | polar solvents | | | |

*Silica gel can be dissolved by solvents containing more than 10% MeOH.

Visualizing TLC plates: Always check TLC plate with UV lamp first (Ultra-Violet Products UVG11). Note any UV active spots. We stain our TLC plates by dipping them in a CAM solution (see below) and heating them on a hot plate (10s – 2min).

CAM TLC stain (Ceric ammonium molybdate)

| | |
|-------|---|
| 5g | Ce(SO ₄) ₂ |
| 25g | (NH ₄) ₆ Mo ₇ O ₂₄ |
| 450mL | water |
| 50mL | H ₂ SO ₄ |