

MALDI sample preparation: the ultra thin layer method

See [larger video](#).

If you can't see the movie, please try installing the newest version of [Quicktime](#).

Necessary reagents and instrumentation:

It is critical that all reagents are of highest-quality (e.g. HPLC grade).

- Powder-free gloves (latex or nitrile)
- Stainless steel or gold MALDI sample plate
- Methanol (MeOH)
- Water
- Kimwipes®
- α -cyano-4-hydroxycinnamic acid (4-HCCA)
- Trifluoroacetic acid (TFA) (final concentration in 0.1% in H₂O)
- Acetonitrile (ACN)
- Formic acid
- Isopropanol

Protocol:

It is very important to wear powder-free gloves during the preparation of the thin layer.

1. Cleaning of the sample plate:
 1. Use a stainless steel or gold MALDI sample plate.
 2. Wash with MeOH and wipe gently with a Kimwipe. Do not rub or scrub the surface with the Kimwipe —you might scratch the surface.
 3. Wash with H₂O and wipe gently with a Kimwipe.
 4. Wash with MeOH and wipe gently with a Kimwipe.
 5. If needed, repeat MeOH/H₂O/MeOH cleaning cycle, always ending with MeOH.
 6. Make sure that there are no ghost spots or residue remaining on the plate. If there are ghost spots, rinse the plate with deionized water while rubbing the surface with your gloved hand (Do not rub with Kimwipes). Repeat the MeOH/H₂O/MeOH cleaning cycle.
 7. Use the plate immediately after washing.
2. Preparation of the thin layer substrate solution:
 1. Mix 1 part of saturated 4-HCCA (2 parts ACN, 1 part water, and 0.1% final TFA) and 3 parts isopropanol.

Notes:

- The thin layer substrate solution is very stable, and it can be kept at room temperature in the dark for a year. It may also be stored at 4°C.

3. Making the thin layer substrate:

0. Apply 20-50mL of the thin layer substrate solution on the left-center of the plate, and spread with the side of a pipette tip. Sweep in one direction.
1. As the solution dries, the organic solvent evaporates very fast leaving behind minute droplets of water on the surface of the plate. At this point wrap your index finger with a ply of Kimwipe and initially gently blot the surface of the plate with it.
2. When the surface is freed from water droplets, wipe the entire plate with uni-directional sweeping motions using a Kimwipe.

Notes:

- If the solution does not spread enough throughout the plate surface, increase the ACN composition and recreate the thin layer. Some parameters that may affect the spreading of the solution include ambient humidity and temperature.
 - With gold plates, the layer usually appears as a yellowish reflection, depending on viewing and light angles. With steel plates, only the edge of the layer is normally visible.
3. Clean the edges of the plate with a fresh Kimwipe before placing it in the plate holder to prevent matrix from contaminating the instrument.

4. Preparation of the matrix solution:

[Saturated 4-HCCA](#) in FWI (3 parts formic acid, 1 part water, 2 parts isopropanol) is recommended for analysis of both membrane and soluble proteins. It might also be used in special cases for the analysis of very hydrophobic large peptides ($M > 4,000u$). Make sure that the matrix solution used for spotting the proteins do not exceed ~50% organic content, because otherwise it will completely dissolve the thin layer substrate and defeat the advantages of the method.

5. Test of the thin layer substrate:

Spot 0.5mL of the matrix solution on the plate. A whitish precipitate will appear at the interface between the thin layer and the droplet. When this layer covers the bottom of the droplet entirely, usually within 10-15 seconds, aspirate the excess liquid with a vacuum line. If it takes longer than 30 seconds for the precipitate to form, it is an indication that there might be a problem with the thin layer substrate and/or the matrix solution.

6. Sample application:

0. The analyte concentration in the starting solutions should be within 10 and 1000mM.
1. Dilute the analyte solution in matrix solution to a final analyte concentration between 0.2 and 2mM. For example, start with 1ml of sample and 9ml of matrix solution.

Notes:

- **Make sure that the matrix is close to being saturated in the final solution, otherwise you will redissolve the thin layer substrate.**
 - Analyte concentrations required to obtain decent signals is highly dependant on solution complexity and composition (e.g., contaminants) and analyte ionizability.
 - If a single-step dilution does not yield good results, you should try another dilution step. Although counter-intuitive, highly concentrated analyte solutions rarely produce good spectra.
 - Membrane proteins usually require larger dilutions.
2. Spot 0.5ml of sample/matrix mixture on the plate. A whitish precipitate will form at the interface between the thin layer and the droplet. This precipitate is the cocrystallization of matrix and analyte. When this layer covers the bottom of the droplet entirely, usually within 10-15 seconds, aspirate the excess liquid with a

vacuum line. If it takes longer than 30 seconds for the precipitate to form, it may be an indication of a contaminant present, which prevents crystallization, or your analyte is too concentrated.

7. Calibrant application:

0. Prepare the calibrant/matrix mixture in a similar manner, with a final calibrant concentration between 0.1-0.5mM.
1. Spot 0.5ml calibrant/matrix mixture on the plate in close proximity to the sample spots. This physical proximity is used in a pseudo-internal calibration procedure in which the plate is moved from the sample spot to the calibrant spot during sample acquisition, to add a few laser shots of the calibrants to the spectrum. This approach guarantees a higher mass accuracy calibration than external calibration only.

8. Washing step:

Wash each spot with approximately 2mL of a ice cold 0.1% aqueous TFA solution. Aspirate excess liquid with a vacuum line.

9. Acquisition of mass spectra:

You are now ready to acquire mass spectra. If you use the FWI matrix solution you must acquire your spectra as soon as possible to prevent irreversible modification to the analyte such as formylation.

References:

F. Xiang, R.C. Beavis, **A Method to Increase Contaminant Tolerance in Protein Matrix-Assisted Laser Desorption Ionization by the Fabrication of Thin Protein-Doped Polycrystalline Films** *Rapid Communications in Mass Spectrometry* 8 (1994) 199-204.

M. Cadene, B.T. Chait **["A Robust, Detergent-Friendly Method for Mass Spectrometric Analysis of Integral Membrane Proteins"](#)** *Analytical Chemistry* 72 (2000) 5655-5658.