

Protocol for sample preparation for mass spectrometry analysis by MALDI-MS

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Chemicals

- » Ammonium hydrogen carbonate
- » Acetonitrile HPLC grade
- » Trypsin ultra grade sequencing (Promega)
- » α -Cyano-4-hydroxycinnamic acid (4-HCCA)
- » 2,5-Dihydroxybenzoic acid (DHB)

Matrix solutions

WMAc H₂O : MeOH : acetic acid (35:60:5 (v/v/v))
WA water : acetonitrile (2:1 (v/v))
TWA WA + 0.1% TFA (final concentration in TWA)
FWI formic acid, water, isopropanol (1:3:2 (v/v/v))

4-HCCA sol'n Dissolve 2 mg 4-HCCA in ~ 800 μ l of pure acetonitrile. (4-HCCA; MW=189.04, characteristic monoisotopic peaks: 164.047, 172.040, 195.050, 212.032, 294.076, 379.093). The solution can be used for about one week in the refrigerator. Use brown Eppendorf tubes for 4-HCCA, since it is light sensitive.

DHB sol'n Dissolve DHB in WMAc (or, alternatively, WA or TWA). Use 1:3 dilution of saturated DHB solution for spotting. (DHB; MW=154.03, characteristic monoisotopic peaks: 137.024, 154.027, 155.034, 273.040)

Destaining and washing of gel spot

1. Destain the gel spot by shaking at 4°C o/n (a few hrs is sufficient depending on time of day)
2. Aspirate off wash solution with gel loading tip, replace with fresh 0.5 ml of wash and shake for 1hr @ 4°C
3. Wash with 200 μ l qH₂O once and with 200 μ l 100 mM NH₄HCO₃ / acetonitrile (1:1, v/v) twice for 15 min.
4. Shrink the gel in 200 μ l 100 % acetonitrile and flick until the gel pieces turn white. Incubate for 5 min.
5. Aspirate off acetonitrile, let air dry for 5 min.

Reduction and alkylation of proteins (skip steps 18-23 if you have alkylated the proteins before running the gel!)

6. Swell the gel spot in 200 μ l freshly prepared 10 mM DTT in 50 mM NH_4HCO_3 .
7. Incubate for 1 hour @ 37°C.
8. Remove the DTT solution and add 200 μ l freshly prepared 55 mM IAA in 50 mM NH_4HCO_3 .
9. Incubate for 1 hour at RT in the dark.
10. Wash two times with 200 μ l 100 mM NH_4HCO_3 / acetonitrile (1:1, v/v) for 15 min.
11. Shrink the gel spot in 200 μ l 100 % acetonitrile.

In-gel digestion

12. To one vial (100 μ g) of trypsin sequencing grade (Promega), add 1 ml of a 1 mM HCl solution. Aliquot 10 μ l and store @ -80°C. Just before use, dilute the trypsin stock solution 10 times in 50 mM NH_4HCO_3 .
13. Add a sufficient amount of the diluted trypsin solution to fully immerse the gel pieces (use about 30 μ l for 3 x 1 mm bands).
14. Leave for 30 min @ RT and then check whether the gel plug is still fully covered by the trypsin solution. If not, add some more of the diluted trypsin solution to fully cover the gel pieces.
15. Incubate overnight at 37°C; shaking not necessary.

Extraction of peptides from the gel and solution

16. Spin down for 10 s.
17. Add 50 μ l of a 0.5 % formic acid solution in 30% acetonitrile; sonicate in a ultrasonic bath for 2 min and then incubate for 30 min @ RT.
18. Transfer the supernatant to a clean eppendorf tube or into a 96 well plate.
19. Repeat steps 29-30 twice.
20. Dry the combined supernatants in a speedvac (Room 681).
21. Label the sample tubes or well plate with the appropriate indexing number (ask Karel orf Jeroen) and store the dry peptides @ -20°C until MALDI-MS analysis.

Plate preparation (for AnchorChip plate: see below)

Clean the Plate (in flow hood!):

22. Take a clean stainless steel plate.
23. Wash with MeOH. Wipe with Kimwipe.
24. Wash with H_2O . Wipe with Kimwipe.
25. If needed, repeat MeOH/ H_2O cycle, ending with MeOH.
26. Use acetone to dry the back of the plate to get rid of H_2O

AnchorChip plate preparation (see also www.bruker-daltonik.de)

OPTIONAL

Thin-layer

(For jump-start crystallization; used for whole-protein or large peptides; skip this procedure when using dried droplet method; compatible with WA, TWA and FWI solvent systems; compatible with 4-HCCA and sinapinic acid matrixes)

- » Thin-layer solution = 1 part of saturated 4-HCCA in TWA + 3 parts isopropanol (very stable, can be kept in dark for a year).
- » Spot 20 μl of the thin-layer solution on the middle of the plate, spread with the edge of a pipet tip over the plate except 1cm from the edge (not used due to edge effect). Try to do this in one sweeping movement. Before it is completely dry, tap all over lightly with a Kimwipe to spread remaining water droplets. Once dry, wipe the entire plate with four sweeping motions with Kimwipe, pressing relatively hard. With gold plate, the layer should only appear as a yellowish/blue reflection, depending on the viewing angle and the light angle. With steel plate, only the layer edge should be visible.

Spot samples on the plate

27. Usually, 50 fmole per protein in 0.5 μl on one spot is a good amount. Also spot peptide calibrants (100 fmole/ μl). Digested peptides – solvent is chosen based on matrix used and hydrophobicity of the peptides.
28. Take 0.5 μl sample or standard and add to 4.5 μl matrix solution. Dilution helps to reduce contamination and incorporate sample into matrix better (Matrix solvent is volatile. Therefore, too little matrix solution will not be enough to dissolve the sample).

Alternative spotting methods

Dry-droplet method	Thin-layer method (for proteins, peptides, WA or TWA solvent is OK)
<p><u>Half-saturated 4-HCCA / TWA matrix solutions</u></p> <ol style="list-style-type: none"> 29. Prepare a matrix solution of 4-HCCA 30. Centrifuge for 5 minutes at 14000 rpm. 31. Transfer the solution to a fresh tube. 32. Dilute the solution 1:1 with TWA. 33. Spot 0.5 μl sample (or 0.25 μl of standard) on the plate. A transparent spot starts forming immediately. 34. Speed-Vac the plate for 2 min. 35. Quickly wash each spot with approx 2 μl of ice-cold 0.1% TFA solution. Suck up excess liquid with vacuum line. 	<p><u>Saturated 4-HCCA / FWI matrix solutions</u> (used within 2 hours of preparation)</p> <ol style="list-style-type: none"> a. Put 120 μl FWI in the Eppendorf tube containing enough dried 4-HCCA to form a saturated solution. b. Scrap the wall of the Eppendorf tube and vortex at max speed. c. Centrifuge for 6 minutes at 14000 rpm. d. Transfer 100 μl supernatant (saturated 4-HCCA) into a fresh tube (be careful not to suck up the powder!!) e. Spot 0.5 μl sample (or 0.25 μl standard) on the plate. f. A whitish opaque spot starts forming immediately. g. When the layer underneath is homogeneous (it usually takes 10-15 seconds), suck up excess liquid with the vacuum line. This will help getting rid of contaminants since usually contaminants are more difficult to crystallize. h. If the opaque spot takes longer than 30 seconds to appear, you have a contaminant that prevents crystallization or your analyte is way too concentrated. i. Wash each spot with approx 2 μl of ice-cold 0.1% TFA solution. Suck up excess liquid with vacuum line.
<p>Heterogeneous crystals. Less noise for small m/z; at large m/z it will have broad peak.</p>	<p>Homogeneous crystals. Less noise for large m/z</p>