MALDI SAMPLE PREPARATION

MATERIALS

10mM Ammonium Phosphate stock solution
Solvents:
0.1% TFA: Water (high purity) with 0.1 % TFA
Acetonitrile (ACN) with 0.1% TFA
50:50: ACN-Water 50:50 with 0.1 % TFA – (See Note 1)
70% ACN: ACN-Water 70:30 with 0.1% TFA
3% ACN: ACN-Water 3:97 with 0.1% TFA

Matrix Solution:

1. Measure 5mg of α-cyano-4-hydroxycinamic acid (CHCA) in HDPE vial or a vial which the plasticizers have been removed with an acetonitrile rinse.
2. Add 950µL of 50:50 to the vial containing CHCA, and 50µL of 10mM Ammonium Phosphate stock.
3. Vortex 1-2 minutes to dissolve, and allow to settle (or briefly centrifuge).

Sample Spotting Solution:
Sample and Matrix should be mixed in a 1:1 ratio for most samples. Vortex the CHCA stock solution for 1 min and centrifuge for 30 sec or let the solution settle for 5-10 min. Take an aliquot, (2-10 µl) usually 10µL, according to final desired concentration and add an equal volume of 50:50. Use this solution to extract peptides from the ZipTip or make dilutions.

EQUIPMENT

- C18 ziptips or Omix tips for peptides OR C4 ziptips for proteins
- 1 Centrifuge tube (no plasticizers) for each sample to elute peptides from tips into.
- Glass waste beaker (small)
- MALDI plate
- 1-10µL eppendorf pipette
- 0.5-1µL eppendorf pipette

Calibration Mix for Matrix Standards:

1. 1:10 Dilution: Add 1uL of 4700 Calibration Mixture (red top) to 9µL of 50:50 and vortex then centrifuge briefly.
2. Standard: Add 1µL of 1:10 dilution to 12µL of Matrix and 12µL of 50:50. Vortex and centrifuge briefly.

Spot Standard on the 6 CAL spots around each plate before loading into the AB4700.

Note 1: Water should be double-deionized, such as Milli-Q grade 18 mΩ. HPLC-grade water can vary in salt content and is not recommended. **Organic solvents** (ACN) should be of the best quality available (HPLC-grade is acceptable). **Vials** and containers used for sample preparation should be free of plasticizers and it is recommended to rinse them prior to use with 50% ACN, rinse, then 1-4% TFA, with rinse.

STORAGE:	HAZARDOUS SUBSTANCES:
Matrix: freeze for overnight, can refrigerate during the day. Only good for 1 week. Solvents: Room Temp. Good unless contaminated or cap is left off. Samples: Uncleaned samples are saved and stored in styrofoam boxes in freezer Eluted Samples: Samples in matrix can be frozen and are good for 1 week, If samples are in 70% ACN they are labeled and frozen with the uncleaned samples.	α-cyano-4-hydroxycinamic acid (CHCA) **ALL SOLVENTS AND ACIDS** (add acid to water)

METHOD

Sample Cleanup with ZipTips (Millipore Cat. # ZTC18S096)**:**

- Clean all centrifuge tubes as described in Note 1. Place an appropriate volume of 0.1% TFA into a separate clean vial for sample cleanup described later (100µL).
- Label a clean centrifuge tube for each sample for peptides to be eluted off of the ziptips into, and place 10µL (or adjusted volume) of CHCA matrix into each tube and cap tightly.

Before use, clean the tips as follows three times for each step using the 1-10µL eppendorf pipette (white): *Take care not to push air through the ziptips at any time*.

1. -10 µl of ACN - discharge solution to waste after each aspiration into pipette
   -10 µl of 50:50 - discharge solution to waste as above
   -10 µl of 0.1% TFA - discharge solution to waste as above

For sample clean up proceed as follows:

2. For dried down trypsin digests:
   - Add 10µL of 3% ACN solution to the sample in a cleaned centrifuge tube.
   - Vortex for 30sec to 1 minute, and make sure liquid is at the bottom of the tube.
   - The sample is now ready for ZipTip cleanup.
3. Draw 10µl of the solution containing the digested protein or peptides several times into the washed tip in step 1, and expel into the same sample vial keeping the tip under liquid at all times. For less protein (or less concentrated bands), pull through ziptip more times.
4. Wash the tip with the bound peptides, 2 to 3 times with 10µl of 0.1% TFA solution from the separate centrifuge tube and discharge the washing solution to a waste beaker to remove salts.
5. The peptides are now clean on the C18 tip. To elute the sample off the tip, place the ziptip into the new sample vial with 10µL of CHCA matrix, and pipette up and down 3 times avoiding any air. Push the last bit of solvent off the tip by raising the tip just above the matrix in the tube and pushing out the remaining air into the sample tube. This will recover 50 to 70% of the sample. Place used ziptips into a ziptip rack labeled as used.

SPOTTING SAMPLES ELUTED WITH CHCA MATRIX:

- Draw 0.7µL of the clean peptides/matrix solution and spot in the wells of a row of the MALDI plate heated over a dry bath to 35-40°C (recommended) and let air dry, or place unheated plate under vacuum.

Note 3: If sample remains oily, this implies that plasticizers have contaminated the sample.

- Each row in the plate is numbered by odd or even numbers for each letter. There are 192 wells for sampling, 6 calibration wells for standards (do not use these wells for samples). If sampling amounts do not require the all wells on the plate, leave well D12 and D14 for reference standards. Indicate separately the identity of each sample (an Excel format sample log sheet is available).
- It is recommended to spot at least 3-4 wells with the same sample and up to a whole row. Try to spot each project on a separate plate or section of a plate.

Make sure a grid is filled out and the last four numbers of the plate barcode are recorded in association with that sample set.

Sample spotting for samples eluted in 70% ACN:

a) Mix sample with matrix 1:1 to spot, and spot over heat as above **OR**
b) Heat the plate to between 35-40°C on a dry bath. Spot 5mg/mL matrix on each spot to be used. Allow to dry. Spot samples on top of matrix spot, and allow to dry.

Note 2: α-cyano-4-hydroxycinamic acid (Fluka Cat. # 70990 is recommended) crystals are a bright light yellow color. A mustard-yellow color indicates the presence of impurities and should be recrystallized. To recrystallize, dissolve the CHCA in warm ethanol, filter and add two volumes of deionized water. Let the solution stand over night in the refrigerator, filter the crystal and wash with cold deionized water. Dry crystals under vacuum protected form light. Divide the sample by putting 5mg/vial and keep in the freezer until needed.