SOP: PP053.1
Modified 05/28/15 by MCL

Ag85a,b,c MALDI-TOF/TOF MS Protocol

Materials and Reagents:

1. Matrix: Sinapinic acid 10 mg/ml in 50%acetonitrile (AcN)/0.1%TFA
   0.0100g sinapinic acid / 1ml buffer
   0.0010g sinapinic acid / 100µl buffer
2. Calibration standard: ProtII with sinapinic acid (Aliquot can be obtained from PMF)
3. MALDI target plate: 384 well Bruker steel plate (note 1)
4. 0.6ml eppendorf tubes
5. MALDI-TOF/TOF MS

Protocol:

1._____ Make a fresh saturated matrix solution at 10 mg/ml.

   a. Tare an empty 0.6 ml-eppendorf tube on the digital balance

   a. Weigh out the proper amount of matrix inside the eppendorf tube

   b. Add the solvent into the matrix to make up the concentration at 10 mg/ml

   c. Bath sonicate 5-10 min until the matrix dissolves well into the solvent

2._____ Spot the calibration standard on the MALDI target plate

   b. Put 1 µl of standard on plate first

   c. Put 1 µl of matrix on top of standard, mix well by pipetting up and down.

3._____ Spot sample (fraction) on the MALDI target plate (note 2)

   d. Put 1 µl of sample on plate *first* (otherwise, the matrix might dry before the mixing occurs)

   e. Put 1 µl of the matrix solution on top of the sample, mix well by pipetting up and down

   f. Allow the mixture to air dry overnight (note 3)

4._____ Shoot the plate on the MALDI (note 4) or submit to facility for analysis and look for the following peaks:

   g. Ag85a = MW of ~31,580

   h. Ag85b = MW of ~30,591

   i. Ag85c = MW of ~31,950

5._____ Thaw and pool all clean Ag85a, Ag85b, and Ag85c fractions.

6._____ Concentrate using amicon ultra-15 30,000 MWCO centrifugal device and wash three times with 10 mM AMBIC (note 5).

7._____ BCA each pool.

8._____ Run 1 and 2 µl of each pool on a gel and silver stain.

9._____ Run 1 and 2 µg of each pool on a gel and western blot (SP011) using CS-90 primary antibody for QC.

10._____ Make aliquots (default quantity = 0.25 mg), lyophilize, and store at -80ºC.

Notes:

1. Keep the target plate in good condition:
   - Be sure there are no big scratches on the surface of the plate
   - Check that the pin at the back is not broken
   - Keep it in a safe place, and do not drop it
   - Clean the target plate as needed:
     - Rinse the plate with a squeeze bottle of solvent
     - Rub with a Kimwipe tissue (or other lint-free tissue) to clean
     - Rinse with deionized water
     - If you see any sample or matrix residue, oil, or fingerprints on the plate, bath sonicate until the residues are gone, rinse the plate with a squeeze bottle of solvent and finally rinse the plate thoroughly in deionized water
     - Allow the plate to dry in an area where it will not be exposed to contaminants and dust
2. Samples and Standard should be plated as shown, with all unknown samples surrounding a standard for calibration purposes.

Sample 1	Sample 8	Sample 7	Sample 9	Sample 16	Sample 15
Sample 2	**Standard**	Sample 6	Sample 10	**Standard**	Sample 14
Sample 3	Sample 4	Sample 5	Sample 11	Sample 12	Sample 13

3. It is important to let these samples dry overnight because some of the samples contain glycerol and will not dry quickly.
4. If using the PMF instrument, use the 'LP_44kDa method' method. DO NOT USE THE MALDI UNACCOMPANIED UNLESS YOU HAVE BEEN TRAINED TO USE IT ALONE.
5. The large membrane size will help to remove any remaining low molecular weight contaminants.