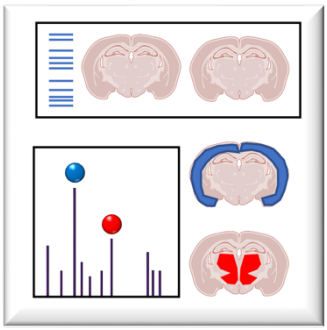
MALDI-IMS Protocols



In Situ Trypsinization for MALDI-IMS

1. Prepare cryosections (10-20 µm thickness) of tissues on ITO glass slides.

2. Delipidate tissues with standard Delipidation Protocol (Carnoy's Wash)

    i. 70 % EtOH for 30 secs
    ii. 100 % EtOH for 30 secs
    iii. Carnoy's Solution for 2 mins.
    iv. 100 % EtOH for 30 secs
    v. 40 % EtOH: 60 % d.H_20 for 30 secs
    vi. 40 % EtOH: 200 mM ammonium bicarbonate (ABC) solution for 30 secs
    vii. 100 % EtOH for 30 secs

    Carnoy's Solution: 6 parts 95 % EtOH, 3 parts chloroform, 1 part glacial acetic acid

    ABC Solution: 200 mM ammonium bicarbonate in d.H_2O

3. Prepare Trypsin Gold (Promega) at a concentration of 500 ng/mL in a 50 mM ABC solution.

4. Spray the tissue with Trypsin Gold solution three times, making sure the surface is coated and remains damp between passes. Do not air dry but proceed directly to incubation step under controlled humidity.

5. Prepare a controlled humidity chamber by placing a glass jar with a sealed lid in a 37°C incubator. Humidity is maintained at 87% in the chamber by adding saturated KNO_3 solution to the bottom of the jar. Place the trypsin sprayed ITO slide on an elevated flat stage above the solution.

6. Place the slide(s) in the humidity chamber, face up and incubate for 15 minutes to overnight.

7. Remove the slide from the humidity chamber and allow it to dry in a desiccator without vacuum for 20 mins.

8. Prepare a saturated solution of α-CHCA matrix in 75% acetonitrile: 25% water and 0.1% trifluoroacetic acid (TFA).

9. Spray the slide three times with the CHCA matrix solution, allowing the surface to dry between each pass.

10. Place in vacuum desiccator for 20 mins prior to transfer to IMS system.