

3 Sample Preparation

3.1 For 5 mm NMR Tubes

Urine

- Carefully thaw the samples, which have been stored at -80 °C, at room temperature (30 minutes for ≤ 2 mL volume sample).
- Centrifuge the samples for 5 to 10 minutes at ~2000 RCF.
- Pipette 100 µL of Bruker urine buffer into a Cryovial or Eppendorf container (of 1.5 mL or 2 mL volume).
- Add 900 µL of urine.
- Mix the buffered urine for 30 seconds on the Vortex mixer.
- Transfer 600 µL of well mixed sample into a 5 mm 7" NMR tube or a 5 mm SampleJet rack tube.

Plasma/Serum

- Carefully thaw the samples, which have been stored at -80 °C, at room temperature (30 minutes for ≤ 2 mL volume sample).
- Pipette 400 µL of Bruker plasma buffer into a Cryovial or Eppendorf container (of 1.5 mL or 2 mL volume).
- Add 400 µL of plasma/serum.
- Shake the mixture gently for 1 minute (do not use the Vortex mixer).
- Transfer 600 µL of well mixed sample into a 5 mm 7" NMR tube or a 5 mm SampleJet rack tube.

CSF (CSF = Cerebrospinal fluid)

- Carefully thaw the samples, which have been stored at -80 °C, at room temperature (30 minutes for ≤ 2 mL volume sample).
- Add 150 µL of Bruker CSF buffer into a Cryovial or Eppendorf container (of 1.5 mL or 2 mL volume). The CSF buffer = 60% Bruker urine buffer + 40% demineralized H₂O.
- Add 750 µL of CSF.
- Shake the mixture gently for 1 minute (do not use a Vortex mixer).
- Transfer 600 µL of well mixed sample into a 5 mm 7" NMR tube or a 5 mm SampleJet rack tube.

MeOH Extract

- Extract samples using a solvent of 4.76 % CD₃OD in MeOH (for instance, 50 mL Methanol-d₄, ≥99.8 atom % D, in 1000 mL GC-MS grade Methanol), and 0.95 % w/v TSP (for instance, 100 mg 2,2,3,3-d(4)-3-(Trimethylsilyl)propionic acid sodium salt, ≥98 atom % D, in 1050 mL MeOH-CD₃OD mixture).
- Transfer the MeOH Extract using a pipette into a 5 mm 7" NMR tube or a 5 mm SampleJet rack tube. The target volume of MeOH Extract inside the tube must be 600 µL. Mind the material loss while transferring.