Protocol: Metabolite Extraction from Cells

Keep samples on ice or cool as possible especially during vortex

Perform all steps in epi-tube (2ml) (30 min)

This protocol assumes you are starting with homogenized tissue or cells.

Washing of cell pellet (can skip if cells have been washed previously):

- Resuspend cell pellet in (50mM NaCl or PBS) and transfer to epi-tube (2ml)
  Keep samples on ice from this point forward when not performing an extraction step.
- Spin at 2,000xg (15 min @ -4 C)
- Remove and discard supernatant

Cell Lysis

Extraction (2X[100%H2O]:[WET CELL PELLET]) (5min/sample)

- Resuspend cell pellet in 100% H2O
  - Cover cell pellet with water
  - Add 2x that volume more water (e.g. if 20 uL covers the cell pellet, then add 40 uL more for a total of 60 uL)
- Vortex at least 30 seconds or until homogenous mixture is murky (use powerful vortexer)
- Sonicate 60% duty cycle power (5min/sample)
  - Samples should remain on ice or with cold zinc beads
- Add equal volume of 100% MeOH
  - (E.g. above: add 60 uL methanol to the 60 uL of H2O)
- Vortex 30s
- Spin at 20,000xg for 15 min (-9C)
- Pool Top MeOH /H2O fraction into a fresh tube: [Metabolite Extract]
- Discard cell debris properly
Acetone Precipitation to remove proteins: (2.5 hours)

• (5:1 v/v) Add 5x volume of -80C Acetone to [Metabolite Extract]
  ▪ (e.g. above: add 5x120 uL = 600 uL acetone to [Metabolite Extract])
• Freeze in -80C for two hours (minimum) or overnight
  ▪ If using samples with a low cell count consider keeping at -80C overnight
• Spin at 20,000xg for 5 min
• Remove metabolite rich supernatant and transfer to fresh tube
• Discard protein pellet properly

Concentration: (2.5 hours)

• Dry in speed vac at 30 C
• Set speed vac for increments of 10-15 minutes and check frequently
• If a gel forms or liquid is gone, remove samples from speed vac
• Store DRY extracts at -80 C until LC-MS analysis
  ▪ avoid long-term storage before analysis
  ▪ samples should be shipped DRY
• Resuspend in 50% MeOH/H2O prior to LC-MS (volume can be adjusted: typical 50-100 uL)
  ▪ Transfer to small volume polypropylene autosampler vial
  ▪ LABEL vials

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