# 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 $H_2O$)
- Vortex 30s
- Spin at **20,000xg** for 15 min (-9C)
- Pool *Top* MeOH /$H_2O$ 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/$H_2O$ prior to LC-MS (volume can be adjusted: typical 50-100 uL)
    - Transfer to small volume polypropelyne autosampler vial
    - LABEL vials



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