Protocol for Harvesting Intracellular Metabolites for GCMS

Purpose: Measure the abundance of intracellular polar metabolites (like amino acids)

SET UP/SUPPLIES

One rectangular ice bucket @ the bench
P1000 pipette and tips
P20 pipette and tips
Serological pipettes and stripettor
Cold methanol – GCMS Grade @ -20C fridge in main lab door
Cold chloroform @ -20C fridge in main lab door
Cold saline (NOT PBS) @ 4C fridge in main lab (large white cardboard box on left side)
Norvaline (2mg/mL) @ -20C fridge in main lab door, small Eppendorf tube or find in "GCMS Reagent" box in Coloff Lab rack of -20C fridge in main lab

Dilute Norvaline

For each well in a 6well plate you need 1uL of (2mg/mL) Norvaline + 300uL autoclaved H2O (ex: one 6well plate needs 6uL Norvaline + 1800uL water total)
Make a Master Mix with enough diluted Norvaline for all wells you will harvest
Example: 18 wells total (make enough for 20) = 20uL Norvaline + 6mL H2O.
Vortex final dilution to mix thoroughly.

BEGIN:

Place 6well plate on ice
Aspirate media
Wash each well w/ 1mL of Saline
Aspirate Saline - make sure wells are completely dry

Add 500uL of GCMS-grade METHANOL to every well

Add 300uL of Norvaline to each well

Scrape the first well, transfer to Eppendorf tube (~800 uL), keep on ice (I like to use a blue metal tube rack that stays super cold in the ice bucket)
You can use the same scraper for replicate wells (a whole row of 3 wells that are the same) to cut down on scraper waste

Option to store samples at -80C BEFORE adding chloroform

Once all wells scraped and collected, add 600uL CHLOROFORM to each tube

Vortex at 4C for 10 minutes (fancy sponge)
Centrifuge at 4C, max speed for 10 minutes

You should see obvious phase separation after centrifugation – top layer contains polar metabolites, thin white border is all protein (Don't touch!), bottom layer is lipids

CAREFULLY transfer top layer to fresh Eppendorf tubes (~450uL) – DO NOT TOUCH PROTEIN LAYER
Air-dry metabolites for ~2 hours (in the fume hood, listen for slow steady air flow and no bubbling in the tubes)

After drying, option to store dried samples at -80C

Continue with derivatization protocol using MOX and TBDMS

Add 15uL of MOX reagent to each tube (do this in fume hood!)

(Mox is found in 4C fridge in main lab, right side, Styrofoam tube rack, the stock bottle of MOX has a sealed top, use a 1mL syringe (Huiping's bench) to extract what you need and store in an eppendorf tube, discard syringe in sharps bin).

Vortex each tube for at least 10 seconds (at the bench)
Heat at 37C for 90 minutes

Add 20uL of TBDMS to each tube (do this in fume hood!)

(TBDMS found in -20C main lab, light blue tube rack 2mL tube, or stock ampules are on top shelf of -20C fridge in long skinny white box, break open in fume hood to refill 2mL tube as needed).

Vortex each tube 10 seconds (at the bench)
Heat at 60C for 60 minutes

Transfer each sample to a GCMS tube – found either in Kelly's bench top drawer or GCMS drawer across from Huiping's desk. Discard all waste (tips and tubes) in a bleach bottle labeled "GCMS Waste"

Load samples into GCMS autosampler