Protein extraction protocol for phosphoproteomics

For protein samples on polycarbonate filters

Developed in the Saito lab by Dawn Moran, modified for phosphoproteomics by Noelle Held

Materials

- SDS extraction buffer (1% SDS, 0.1M Tris HCL, pH 7.5, 10mM EDTA)
- Ethanol clean 2mL microfuge tubes, 4 per sample
- 50/50 Methanol: Acetone solvent mixture (cold)

Preparation

- Prepare extraction buffer

Protocol

- Perform all steps on ice unless otherwise noted
- Carefully unfold filter and arrange in a clean 2mL tube to maximize surface area exposed to solvent
- Add 1.5mL SDS extraction buffer and incubate at RT 15mins
- Heat 95C for 10minutes
- Shake RT 350RPM 1hr
- Decant extract into a new 2mL tube and centrifuge high speed for 20mins at room temperature
- Remove supernatants, carefully leaving the pellet (debris) in the original tube; retain the pellet until samples are run just in case extraction was not successful
- Concentrate supernatants in 6mL Vivaspin columns to approximately 300uL (use your judgement about concentration of the sample)
- Place the sample in a clean 2mL tube and add 50:50 solvent mixture in a 1:4 ratio.
- Leave samples overnight at 20C or longer to precipitate the proteins. START:
- At end of precipitation period, centrifuge the precipitated protein mixture to collect the proteins, 30mins at 4C high speed
- Remove supernatant to another clean tube and place back in freezer until samples are run (proteins may continue to precipitate)
- Dry proteins by speedvac about 10mins
- Resuspend pellets in minimal SDS buffer at room temperature (shaking may be needed) - about 1hr to totally dissolve
- Quantify the proteins with the Bio-RAD DC protein assay