K. Mace, September 2007

Urea Lysis Protein Purification from Tissue

Prep: Homogenizer, with 2 100 mL grad cylinders for rinsing
Ice bucket with powdered dry ice
Falcon 2059 tubes (14 mL)
Scalpel and glass plate

Stock Buffer – (make 900 mL: DO NOT AUTOCLAVE)

100 mM NaH_2PO_4 → 13.8 g NaH_2PO_4-H_20 (MW 137.99)
10 mM Tris-Cl → 1.2 g Tris base (MW 121.1)
8 M Urea → 480.5 g Urea (MW 60.6)

NOTE: Stock Buffer is 900 mL instead of 1 liter so to allow for volume increase with pH adjustment, so for each buffer below, start with 18 mL and bring volume up to 20 mL.

Urea Lysis Buffer (make 20 mL/sample from Stock Buffer)

→ Adjust pH to 8.0 using NaOH immediately prior to use

1. Keep samples on dry ice until ready, weigh sample in tube
2. Roughly mince tissue with clean scalpel on clean glass plate
3. Place pieces into Falcon 2059 tube and add 1 mL Urea Lysis Buffer/200 mg tissue.
4. Homogenize sample 3X-20 seconds, rest in between each sample (check for completion).
5. Optional: spin 10 minutes in Sorvall at 12 Krpm, transfer supe to new tube. Set aside 15 uL for protein assay.
6. Aliquot samples to small tubes (0.6 mL) snap freeze on dry ice and store at -80°.