Human Lung Tissue Extraction Protocol

- Anabel Gonzalez & Huifeng Yu

Materials & Reagents:

PART I-

- Guanidinium Buffer: 6 M guanidinium hydrochloride, 5 mM EDTA, 10 mM monosodium phosphate, adjusted to pH 6.5 with NaOH. May be stored at ambient temperature (RT); cool to 4˚C prior to use.
- Potter-Elvehjem homogenizer
- 100 mM phenylmethylsulfonyl fluoride (PMSF) in dimethylsulfoxide (DMSO)
- Petri Dishes
- 5-ml Eppendorf tubes
- Liquid nitrogen
- Mortar and pestle

PART II-

- Dialysis Cassette (e.g. Thermo Slide-A-Lyzer MINI #69572)
- Dialysis Buffer: 1M Urea, 20mM sodium Phosphate, pH 7.4

PART I: Tissue Processing-

Method 1: Mouse Lung (Trachea, Bronchus and Parenchyma), Brain and Heart

1. Weigh tissue. All subsequent steps are on ice unless indicated.
2. Transfer tissue to Potter-Elvehjem homogenizer.
3. Add PMSF to Guanidinium Buffer at 1:1000 (final 0.1 mM PMSF).
4. Add Guanidinium Buffer (with PMSF) at 1 ml per 0.1 g of wet tissue.
5. Homogenize the tissue on ice until uniform (~10 strokes)
6. Mix end-over-end 5 h - overnight at 4˚C
7. Centrifuge 20,000 x *g*, 4˚C, 30 min. If particles remain in suspension repeat for 15 min.
8. Discard pellet, keep supernatant = “mLuG” “mBrG” and “mHrG”

Method 2: Human Trachea/Bronchus and Parenchyma [supplied as extract]

1. Weigh tissue. All subsequent steps are on ice unless indicated.
2. Cut human trachea/ bronchus and parenchyma (separately) tissue into small pieces and transfer to a ceramic mortar. Immediately add liquid nitrogen to cover the tissue. Grind to a fine powder using a pre-chilled pestle.
3. Transfer the pulverized tissue to a tarred 5-ml plastic centrifuge tube and weigh.
4. Add Guanidinium Buffer (with PMSF, see above) at 1 ml per 0.1 g of pulverized tissue.
5. Homogenize, mix, then centrifuge as above (mouse steps 5-7).
6. Discard pellet, keep supernatant = “hTrG” and “hPcyG”

PART II: Human and Mouse tissue extract processing for high molecular weight (HMW) protein resolution-

9. Transfer 100 μL of the 6 M Guanidinium Buffer human tissue extract ("hTrG" & "hPcyG") and mouse tissue extract ("mArG" "mBrG" & "mHrG") into the mini-dialysis button. Place the mini-dialysis button into a beaker containing 1M Urea-Phosphate buffer, pH 7.4
10. Let the tissue extract dialyze overnight at 4°C.
11. Retrieve and note of the volume. Centrifuge 20,000 x *g*, 4˚C, 20-30 min. If particles remain in suspension repeat for additional 10 min.
12. Discard pellet, keep post-dialysis supernatant = "D-mArG" "D-mBrG" & "D-mHrG" along with "D-hTrG" & "D-hPcyG"