

Human Lung Tissue Extraction Protocol

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Materials & Reagents:

- Liquid nitrogen
- Mortar and pestle
- Dounce homogenizer
- Detergent Lysis Buffer: 20 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1.0 mM CaCl₂, 2.0 mM MgCl₂, 0.3% CHAPS, 1% NP-40, protease inhibitors (1:1000 dilution of Sigma P8340). Store at 4°C.
- 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.
- 100 mM phenylmethylsulphonyl fluoride (PMSF) in dimethylsulfoxide (DMSO)
- Dialysis Cassette (e.g. Thermo Slide-A-Lyzer MINI #69572)
- Cell Scraper (e.g. Sarstedt 83.1830)
- Petri Dishes
- 5-ml Eppendorf tubes

Method 1: Trachea/Bronchus

1. Weigh tissue. All subsequent steps are on ice unless indicated.
2. Immerse a piece of trachea or bronchus (~0.2-0.3 g) in 0.5 ml of Detergent Lysis Buffer in a Petri dish. Scrape the inner surface with a cell scraper.
3. Transfer extract to an Eppendorf tube, repeat step 1.
4. Centrifuge the combined detergent extracts (~ 1 ml) in an Eppendorf centrifuge (20,000 x g, 5 min). Discard pellet, keep supernatant = "TrSD".
5. Concurrent with Step 4, cut remaining tissue into small pieces add transfer to a ceramic mortar. Immediately add liquid nitrogen to cover the tissue and grind to a fine powder.
6. Transfer the pulverized tissue to a tarred 5-ml plastic centrifuge tube and weigh.
7. Add PMSF to Guanidinium Buffer at 1:1000 (final 0.1 mM PMSF).
8. Add Guanidinium Buffer (with PMSF) at 1 ml per 0.1 g of pulverized tissue.
9. Mix end-over-end overnight at 4°C
10. Centrifuge 20,000 x g, 4°C, 30 min. If particles remain in suspension repeat for 15 min.
11. Discard pellet, keep supernatant = "TrDG"

Method 2: Parenchyma

1. Weigh parenchyma
2. Cut into small pieces in a small Petri dish
3. Add Detergent Lysis Buffer (1 ml per 0.1 g tissue) and transfer to an ice-chilled Potter-Elvehjem homogenizer
4. Homogenize the tissue on ice until uniform (~10 strokes)
5. Centrifuge the homogenate (20,000 x g, 5 min).
6. Discard pellet, keep supernatant = "PcyD".