

Tissue Lysate Preparation Protocol

1. Weigh tissue sample in a 50 mL tube.
2. While keeping sample on ice, wash with cold 1X PBS and aspirate off PBS.
3. Repeat until wash buffer appears clear.
4. Add sufficient volume of cold lysis buffer with 1X PIC, enough to cover sample (about 3 times the weight of sample in volume; i.e. 500 mg sample will receive 1.5 mL lysis buffer). *Note: RNase/ DNase 0.02 mg/mL may also be added to lysis buffer to help facilitate RNA and DNA digestion.
5. Grind/homogenize tissue in tube and incubate on ice for at least 15-60 min.
6. Transfer mixture to microcentrifuge tubes and spin at 14,000 rpm for 30 min at 4°C.
7. Poke through lipid layer and remove supernatant (this is the lysate). Discard cellular debris and lipids.
8. If necessary, respin supernatant at 14,000 rpm and repeat Step 7 to obtain clean lysate free of lipid and debris.
9. Determine protein concentration using the Bradford Protein Assay.
10. Combine equal volumes of 2X SDS sample buffer and cell lysate supernatant. Pipet up and down or vortex several times to mix. Aliquots can be stored at -20°C or -80°C. Prior to use, heat samples at 95-100°C for 3-5 min and then load immediately on SDS-PAGE gel. Avoid freeze/thawing lysate as much as possible.

1X Lysis Buffer Composition (store at 4°C)

- 10 mM Tris pH 8.0
- 130 mM NaCl
- 1% Triton-X100
- 10 mM NaF
- 10 mM NaPi pH 7.5 (sodium phosphate)
- 10 mM NaPPi pH 7.5 (sodium pyrophosphate)

These are added right before use

- 0.02 mg/mL RNase
- 0.2 mg/mL DNase
- 1 mM PMSF
- 1X Protease inhibitor cocktail (PIC)

100X PIC

- 1.6 mg/mL Benzamidine HCl
- 1.0 mg/mL Phenanthroline
- 1.0 mg/mL Aprotinin
- 1.0 mg/mL Leupeptin
- 1.0 mg/mL Pepstatin A

Dissolve in 100% ETOH and store at -20°C. Add 1 ul of 1X PIC for every 100 ul of lysis buffer.

1X PBS

- 137 mM NaCl
- 2.7 mM KCl
- 4.3 mM Na₂ HPO₄
- 1.47 mM KH₂PO₄
- Adjust to final pH of 7.4

Note: This protocol is provided as a guide only. It is used at IMGENEX to test the product development. However, IMGENEX does not guarantee success of an experiment using this protocol. It may be necessary by the researcher to modify the protocol according to a specific project.