

Cell Lysate Preparation Protocol

BUFFERS

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.

1X Lysis Buffer: 10 mM Tris, pH8.0, 130 mM NaCl, 1% Triton X-100, 10 mM NaF, 10 mM NaPi (sodium phosphate), 10 mM NaPPi (sodium pyrophosphate)

2X SDS Sample Buffer: 120 mM Tris-Hcl (pH 6.8), 20 mM EDTA, 4% SDS, 0.06% Bromophenol Blue, 20% glycerol, 0.4% beta-mercaptoethanol

***RNase:** 0.02 mg/mL (Optional, used for RNA digestion)

***DNase:** 0.02 mg/mL (Optional, used for DNA digestion)

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 stored at -20°C (Add 1X PIC to cell lysis immediately prior to use).

FOR MONOLAYER CELLS

Rinse monolayer cells 3-4 times with PBS. On the final rinse, aspirate as much PBS as possible and add 5 ml of ice-cold PBS containing 0.5 mM EDTA and use a cell lifter or cell scraper to bring cells into suspension. Transfer suspension into a 50 ml centrifuge tube and add 5 ml ice-cold PBS to flask. Centrifuge cells at 1,500 rpm for 10 min at 4°C and aspirate supernatant.

FOR SUSPENSION CELLS

Centrifuge suspension at 1,500 rpm for 10 min at 4°C and aspirate supernatant. Re-suspend pellet in 15 ml PBS and centrifuge at 1,500 rpm for 10 min at 4°C and aspirate supernatant. Repeat 2 more times.

1. For every 1 x 10⁶ cells add approximately 100µL of ice-cold Lysis Buffer with 1µL 100X PIC. Resuspend pellet, ensuring no clumps remain. Vortexing or pipeting up and down may be necessary to break up the pellet thoroughly. *Note: RNase/DNase 0.02 mg/mL may also be added to lysis buffer to help facilitate RNA and DNA digestion.
2. Incubate on ice for 15-60 min. After incubation transfer the lysate to one or more microcentrifuge tubes.
3. Centrifuge at 14,000 rpm for 30 min at 4°C. This step separates the lysate into two fractions, soluble (supernatant) and insoluble (pellet).
4. If necessary, respin supernatant at 14,000 rpm and repeat Step 3 to obtain clean lysate free of lipid and debris.
5. Determine protein concentration using the Bradford protein assay.
6. 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.

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.