

Preparing Cell Lysates for ELISA

1. Remove media and rinse cells once with ice-cold PBS.
2. Remove PBS and add 1 ml ice-cold 1X Cell Lysis Buffer (PBS pH7.2, 0.5% Tween-20, 1 mM EDTA) plus 1 mM phenylmethylsulfonyl fluoride (PMSF) to each plate (10 cm in diameter).
3. Scrape cells off the plate and transfer to an appropriate tube.
4. Sonicate lysates.
5. Microcentrifuge for 2 minutes and transfer the supernatant to a new tube. The supernatant is the cell lysate. Store at -80°C in single-use aliquots.
6. Add 100 μl of each diluted cell lysate to the appropriate well for ELISA assay.