

Cell lysis buffer

1. Prepare the components of the lysis buffer on ice and keep the buffer on ice or in the refrigerator once prepared.
2. Cell extraction buffer base (Life Technologies FNN0011) is stored at -20°C. Thaw on ice. Buffer is stable for 2-3 weeks at 2-8°C or for up to year as aliquots stored at -20°C.
3. Add to Cell Extraction Buffer (Life Technologies FNN0011):
 - 1:100 Protease Inhibitor Cocktail (Sigma #P8340)
stored at 4°C, DMSO solution is crystalline at 4°C and melts at room temp.
 - 1:100 Phosphatase Inhibitor 3 (Sigma #P0044)
stored at 4°C, DMSO solution is crystalline at 4°C and melts at room temp.
 - 1:100 Phosphatase Inhibitor 2 (Sigma #P5726)
stored at 4°C, aqueous solution is liquid at 4°C
 - 1:50 PMSF (phenylmethylsulfonyl fluoride, protease inhibitor) stock for working concentration of 1 mM
*50 mM stock; solution prepared in pure ethanol or IPA
stored at -20 °C, sensitive to light (covered in aluminum foil)*
4. General rules of thumb:
 - Use 100 µL lysis buffer per well in a 6-well plate.
 - Use 500 µL per 10 cm plate.
 - Always prepare ~10% extra volume.

Components of Life Technologies Cell Extraction Buffer (FNN0011)

10 mM Tris, pH 7.4, buffer salt
100 mM NaCl, sets overall ionic strength of buffer
1 mM EDTA, ethylenediaminetetraacetic acid, metal chelator
1 mM EGTA, ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid, metal chelator
1 mM NaF, sodium fluoride, serine/threonine phosphatase inhibitor
20 mM Na₄P₂O₇, sodium pyrophosphate, serine/threonine phosphatase inhibitor
2 mM Na₃VO₄, sodium orthovanadate, tyrosine phosphatase inhibitor
1% Triton X-100, nonionic detergent
10% glycerol, stabilization of proteins
0.1% SDS, sodium dodecyl sulfate, anionic detergent
0.5% deoxycholate, anionic detergent

Components of protease inhibitor (P8340), quoted from Sigma website:

This mixture contains individual components, including AEBSF at 104 mM, Aprotinin at 80 µM, Bestatin at 4 mM, E-64 at 1.4 mM, Leupeptin at 2 mM and Pepstatin A at 1.5 mM. Each component has specific inhibitory properties. AEBSF and Aprotinin act to inhibit serine proteases, including trypsin, chymotrypsin, and plasmin amongst others. Bestatin inhibits aminopeptidases. E-64 acts against cysteine proteases. Leupeptin acts against both serine and cysteine proteases. Pepstatin A inhibits acid proteases.

Components of phosphatase inhibitor 3 (P0044), quoted from Sigma website:

This mixture contains individual components with specific inhibitory properties. Cantharidin inhibits protein phosphatase 2A. (-)-p-Bromolevamisole oxalate inhibits L-isoforms of alkaline phosphatases. Calyculin A inhibits protein phosphatases 1 and 2A.

Components of phosphatase inhibitor 2 (P5723), quoted from Sigma website:

This mixture contains individual components with specific inhibitory properties. Sodium orthovanadate inhibits a number of ATPases, protein tyrosine phosphatases, and other phosphate-transferring enzymes. Sodium molybdate inhibits acid and phosphoprotein phosphatases. Sodium tartrate inhibits acid phosphatases. Imidazole inhibits alkaline phosphatases.