

Immunoprecipitation (IP) lysis buffer

1. Prepare the components of the IP lysis buffer on ice and keep the buffer on ice or in the refrigerator once prepared.
2. Lysis buffer base (Cell Signaling Technologies 9803) is stored at -20°C. Thaw on ice. 10X buffer is stable for 1-2 weeks at 2-8°C or for up to 24 months stored at -20°C.
3. Add to lysis buffer base (CST 9803):
 - 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.
 - Remember to account for volume of IP lysis buffer needed to complete IP steps.
 - Always prepare ~10% extra volume.

Components of CST Lysis Buffer (9803), from CST website

20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM Na₂EDTA, 1 mM EGTA, 1% Triton, 2.5 mM sodium pyrophosphate, 1 mM beta-glycerophosphate, 1 mM Na₃VO₄, 1 µg/ml leupeptin

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.