

RIPA buffer for mammalian cell lysis:

50mM Tris-cl pH 7.4

150mM NaCl

1% NP40

0.25% Na-deoxycholate

1mM PMSF

1x Roche complete mini protease inhibitor cocktail

1x Pierce phosphatase inhibitor cocktail

Stock solutions used:

100mM Tris-Cl pH 7.4, 300mM NaCl

10% NP40 in water

10% Na-deoxycholate in water (stored at RT in dark)

200mM PMSF in isopropanol

Roche complete protease inhibitor cocktail tablets (cat# 04 693 124 001)

Pierce phosphatase inhibitor cocktail (100x) (cat # 78420)

For 10ml of lysis buffer:

5ml 100mM Tris-Cl pH 7.4, 300mM NaCl

1ml 10% NP40 in water

3.65ml water

250ul 10% Na-deoxycholate in water

1 tablet* Roche complete protease inhibitor cocktail

100ul* Pierce phosphatase inhibitor cocktail

50ul* 200mM PMSF in isopropanol

*add at time of use. PMSF is only good for ~30minutes after dilution into buffer.