

Preparation of Lysis Buffer

50 mM Hepes pH 8.5, 8 M urea, 1 mM NaF, 1 mM sodium orthovanadate, 10 mM sodium pyrophosphate, 1 mM beta-glycerophosphate

9 M deionized urea (250 mL)

Add 135 g of urea to 250 mL of water. Stir to dissolve.

Add 1 g of Amberlite pellets for each 100 mL of solution. Mix for 10 min at RT. Filter.

Lysis Buffer

Mix the following:

222.2 mL of 9 M deionized urea

10.5 mg NaF

3.21 mL of 78 mM sodium orthovanadate (stock made up using Cell Signaling's protocol)

54.0 mg beta glycerophosphate

1.115 g sodium pyrophosphate

1.30 g HEPES

Stir to dissolve, and pH to 8.5 with 1 M HCl and set the final volume to 250 mL.

Freeze at -80 in 10mL or 50 mL aliquots in Falcon centrifuge tubes (good for 6 mo)