Cytoplasmic and Nuclear Extraction

10x $P_{10}E$:

11.41g/L $Na_2HPO_4 \cdot 3H_2O$
2.79 g/L EDTA

Lysis Buffer:

1mL	10x $P_{10}EG$
2mL	50% Glycerol
50-100uL	Triton-X 100 (75uL for BU-11 cells)
6.6 mL	dH_20

Before use add for 10 mL

10uL	NaOrthovanadate (10mg/mL)
10uL	DTT
45uL	Sigma Protease Inhibitor Cocktail
50 uL	100mM PMSF

Nuclear Extraction Buffer:

	for 50mL
20mM Hepes (pH 7.9)	10mL of 100mM
420mM KCL	8.4mL of 2.5M
1.5mM $MgCl_2$	75uL of 1M
0.2mM EDTA	20μL of 0.5M
20% Glycerol	10mL of 50%

Before use add for 1mL

5uL DTT (100mM)
1uL NaOrthovanadate (10mg/mL)
2.5uL PMSF (20mM)
5uL Sigma Protease Inhibitor Cocktail

1. Collect cells and wash 1x with PBS
2. Decant supernatant and remove remainder with pipet-cells can be frozen here or extracted right away.
3. Add 200-500uL $P_{10}EG$/sample (volume depends on cell type). For BU-11 or Wehi cells pipet up and down about 100x. Breast cancer cells and BMS2 can be broken with Dounce Homogenizer.
4. Place homogenate in epi tube.
5. Spin at max speed for 2 min at RT
6. Transfer supernatant to epi tube (cytoplasm) and save at -80^{o}C.
7. Add 50-100uL Nuclear Extraction Buffer (depends on original cell number) and vortex.
8. Incubate on ice for 30 minutes, vortexing periodically
9. Spin at max for 15 min at 4^{o}C.
10. Transfer supernatant to new epi tube (Nuclear) and store at -80^{o}C.
11. Do Bradford Assay on Proteins.