NUCLEAR EXTRACTION (from cells)

1mM DTT
1X PIC (Protease Inhibitor Cocktail)
10uM Lactacystin
Extraction Buffer
Lysis Buffer
PBS

1) Add DTT, PIC, and lactacystin to lysis buffer.
2) Aspirate media.
3) Rinse cell with ice cold PBS (1x)
4) Add 1mL of lysis buffer / 10cm plate.
5) Scrape cells.
6) Pipette cell lysates into 1.5mL eppendorf tube.
7) Spin at 14K rpm for 1min at 4 °C.
8) Collect pellet (nuclear extract) or Supernatant (cytoplasmic extract) as desired.
9) Resuspend cells in 100ul of ice cold lysis buffer – mix by pipetting up and down.
10) Incubate on ice for 10min.
11) Spin cells @14K rpm for 10min. in a microfuge.
12) Separate supernatant (cyto) from pellet (nucleus).
13) Add DTT and PIC, and lactacystin to extraction buffer.
14) Add 80ul of extraction buffer into the pellet.
15) Resuspend by mixing up and down with pipette.
16) Shake @ slow pace for 10min. so the liquid goes up and down using the shaker in Welch's deli.
17) Spin @ 14K rpm for 10min. using a microfuge.
18) Separate pellet (DNA) from supernatant (nuclear protein).
19) **Supernatant is now your nuclear lysate!!**
20) Flash freeze lysate and store at -70 °C.

NUCLEAR EXTRACTION BUFFER RECIPES

Lysis Buffer (200ml total)

1M Hepes (2ml)

3M KCl	(666.67ul)
0.25M EDTA	(80ul)
10% NP40	(8ml)
dd H20	(189.25ml)

Extraction Buffer (200ml total)

1M Hepes	(4ml)
3M NaCl	(26.66ml)
0.25M EDTA	(800ul)
dd H20	(168.54ml)