1. MATERIALS PROVIDED

- 10X Buffer A Stock (3.0 ml)
  \(100 \text{ mM HEPES, pH 7.9; 100 mM KCl; 100 mM EDTA}\)

- 5X Buffer B Stock (2.0 ml)
  \(100 \text{ mM HEPES, pH 7.9; 2M NaCl; 5mM EDTA; 50\%Glycerol}\)

- 10% IGEPAL (2ml)
- 100 mM DTT (400 ul)
- Protease Inhibitor Cocktail (300 ul)
- 20X PBS Stock (25 ml)

2. NUCLEAR EXTRACTION PROTOCOL

Before you start: Prepare 1X Buffer A from the 10X Buffer A Stock; Prepare 1X Buffer B from the 5X Buffer B Stock; and Prepare 1X PBS from the 20X PBS Stock. Starting with \(10^7\) cells or 0.5 mg of tissue, your yield should be 300-500 ug.

A. STARTING FROM CELL CULTURE (20 RXN)

(See section B for whole tissue nuclei preparation)

The following protocol is optimized for about \(10^7\) cells (near confluent 100mm-plate). Note: All components must be kept on ice at all times.

1. Wash plates with 10 ml of 1X PBS twice.

2. Prepare Buffer A Mix. In a clean, sterile 1.5 ml-Eppendorf tube, add the following (for each plate):
   - 1X Buffer A 1ml
   - 100 Mm DTT 10 ul
   - Protease- Inhibitor Cocktail 10 ul
   - 10\% IGEPAL 40ul

3. Add 1.0 ml of Buffer A Mix (from Step 2) to each plate. Put the plates on ice in an ice bucket and shake at 150 rpm on rocking platform for 10 min.

4. Scrape cells with a sterile scraper, then pipet up and down several times to disrupt the cell clumps.

5. Centrifuge at maximum speed (15000×g) for 3 min at 4°C.

6. Place tubes on ice.

7. Save supernatant (Cytosolic fraction) if desired. Otherwise, discard the supernatant.

8. Prepare Buffer B Mix. In a clean, sterile 1.5-ml Eppendorf tube. Add the following (for each pellet):
   - 1X Buffer B 147 ul
   - Protease Inhibitor Cocktail 1.5 ul
   - 100 mM DTT 1.5 ul
9. Resuspend Pellet in 150 ul of Buffer B Mix. We recommend resuspending the pellet by vortexing at the highest setting for 10 sec. or using a Dounce homogenizer (VWR, cat. # KT885300-0002). Then, lay the Eppendorf tube horizontally on ice in an ice bucket and shake on a rocking platform at 200 rpm for 2 hr.

10. Centrifuge at maximum speed (1500 xg) for 5 min at 4°C.

11. If high salt concentration is a concern, continue to step 12, otherwise proceed to step 14.

12. (Optional) Collect the clear supernatant and dialyze against 50 volumes of Buffer C Mix for 5 hr, to overnight.

12.1. Prepare Buffer C Mix. Except for DTT, the reagents for this buffer are not included in the kit.

- 20 mM Tris, pH 7.9
- 20% (w/v) glycerol
- 0.1 M KCl
- 0.2 mM EDTA
- 0.5 mM DTT

13. (Optional) Spin the dialysate at maximum speed (15,000 xg) for 20 min at 4°C.

14. Collect the supernatant- this is your nuclear extract. Aliquot 5 ul of each sample, and measure protein concentration (e.g., using the Bradford). Store at -80°C or freeze in liquid nitrogen until use.