Nuclear Extract Prep (Moore Lab)

Cells from National Cell Culture Center- 20L prep. Request early passages and early am delivery

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Buffer A = 10 mM Tris pH7.9 (4 deg C), 10 mM KCl, 1.5 MgCl2, 0.5 mM DTT (add before using).
Buffer B = 0.3 M Tris pH7.9, 1.4 M KCl, 0.03 M MgCl2
Buffer C = 20 mM Tris pH7.9, 0.02 M KCl, 1.5 mM MgCl2, 25% glycerol, 0.5 mM DTT & 0.2 mM PMSF (add before using)
Buffer D = 20 mM Tris pH7.9, 1.2 M KCl, 1.5 mM MgCl2, 25% glycerol, 0.5 mM DTT & 0.2 mM PMSF (add before using)
Buffer E = 20 mM Tris pH7.9, 0.1 M KCl, 0.2 mM EDTA, 20% glycerol, 0.5 mM DTT (add before using).

Stocks- 1M Tris pH7.3 at RT, 2M KCl, 1 M MgCl2, 0.5 M EDTA, 1M DTT, 7 mg/ml PMSF (40 mM). All stocks, filtered with 0.45 um filter. Tris came from adding 1M Tris-base to 1M Tris-HCl.

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1. Resuspend cells in 5x packed cell volume Buffer A in four 50mL Falcon tubes (remember to add DTT).
2. It can take awhile to resuspend. Centrifuge at 3K for 5 min. in clinical centrifuge at 4 deg C. Discard the supernatant.
3. Resuspend in 2x packed cell volume and swell for 10 min. on ice.
4. Break cells with ~95 strokes in large (40 ml) glass Dounce homogenizer- "tight" type pestle. Keep the dounces in ice while doing the strokes. Check cells under microscope for 80-90% lysis. Stain cells with trypan blue. Nuclei of lysed cells tend to clump together. Checked cells at 50 and 80 dounce strokes.
5. Spin at 4K for 15 min. in two 50mL Falcon tubes. The loose pellet is the nucleoplasmic fraction. The supernatant is the cytoplasmic fraction.
6. For S-100 prep, take the above supernatant, measure it's volume and add 0.11 volumes of Buffer B. Centrifuge this for 1 hr. at 42K in 55Ti-sw rotor. Dialyze the supernatant against Buffer E. (Instead, we usually add Buffer B and freeze the supernatant in a 50 mL Falcon tube at –80 C in case someone actually wants it in the future)
7. Take the nuclear pellet and measure its volume. Add 0.5 volumes of Buffer C. Gently resuspend the pellet with a pipette.
8. Put this with a small stir bar in a 100 ml beaker surrounded by ice. While stirring, add dropwise 0.5 volumes of nuclear pellet of Buffer D (same amount as buffer C). Adding this too quickly will cause stuff to goo up, we didn't see too much of this. Turns very snotty due to the DNA being released
9. Stir for 30 min.
10. Spin at 10K for 30 min. in two Oakridge tubes in pre-chilled JA-20 rotor.
11. Dialyze the supernatant in 50 volumes or more of Buffer E for 2.5 hours using two 10 Kd MW cutoff Pierce Slide-A-Lyzer (3-15mL volume cassette)-10ml in each, make sure you can see the needle on the other side! Change buffer and go another 2.5 hours. We have seen a lot of precipitate in the cassettes on occasion. If this happens, check the pH of Buffer A and E- We make it up at pH7.3 at room temp, expecting it to be at pH7.9 at 4 deg C, but it should be checked.
12. Example: 20 mL =Supernatant volume
13. 1.5L =Buffer E volume for first dialysis 1.5L =Buffer E volume for second dialysis
14. Spin at 10K for 20 min. in Oakridge tubes in pre-chilled JA-20 rotor.
15. Aliquot in 100ul and 200ul volumes using the multi-pipettor. Freeze on dry ice and store at –80 deg C.