Xiaobing
Gozani Lab
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Small scale Nuclear Protein Extraction without the use of a detergent

*Note: This Sigma procedure requires at least 100 µl of PCV. Use of a syringe is recommended for small-scale preparations **(0.1-1 ml)**. Passage of more than one millliliter through a syringe may cause difficulties due to the needle gauge size.*

1. Scrape the cells using fresh PBS into an appropriate conical centrifuge tube. Centrifuge for **5 minutes at 450 x *g***.
2. Estimate the packed cell volume (**PCV**), e.g. 200 µl.
3. Add 1 ml (**5X PCV**) of 1X Lysis Buffer (including DTT and protease inhibitors) to 200 µl of PCV. Resuspend the cell pellet gently. Avoid foam formation.
4. Incubate the packed cells in lysis buffer on ice for **15 min**, allowing cells to swell.
5. Centrifuge the suspended cells for 5 minutes at 420 x *g*.
6. Resuspend the packed cells in 400 µl (**2X PCV**) of the 1X Lysis Buffer.
7. Cell disruption.
   a. Using a glass tissue homogenizer, transfer the cells into a glass tissue grind tube. Grind on ice slowly with five up-and-down strokes using a type B pestle. Avoid foam formation. OR
   b. Using a syringe with a narrow-gauge (No. 27) hypodermic needle, fill the syringe with 1X Lysis Buffer. The syringe plunger is used to displace the buffer as fully as possible. This removes all the air from the syringe and prevents excess air being pumped into the cell suspension during lysis. Draw the cell suspension slowly into the syringe and then eject with a single rapid stroke. Repeat five times.
   Note: The number of strokes needed varies between cell lines. Start with 5 strokes and then check lysis under the microscope. Lysis should be 80-90%. Lysis can be observed by the addition of the Trypan Blue solution to an aliquot of cells. The dye is excluded from the intact cells, but stains the nuclei of lysed cells.
8. Centrifuge the disrupted cells in suspension for **20 min at 10,000–11,000 x *g***.
9. Transfer the supernatant to a fresh tube. This fraction is the cytoplasmic fraction.
10. Resuspend the crude nuclei pellet in ~140 µl (**2/3X PCV**) of Extraction Buffer (0.42 mM salt) containing the DTT and protease inhibitors. If the procedure is being performed with a homogenizer, it is recommended to give 10 more strokes at this point.
    Note: The salt concentration in the Extraction Buffer is 0.42 M, a commonly used extraction condition. In rare cases a lower or a higher salt concentration may be needed for a better extraction of a particular protein. In that case, dilute the Extraction Buffer with the 1X Dilution and Equilibration Buffer or add NaCl to the Extraction Buffer to reach the desired salt concentration.
11. Shake gently for **30 minutes**.
12. Centrifuge for **5 minutes at 20,000-21,000 x *g***.
13. Transfer the supernatant to a clean, chilled tube.
14. Snap-freeze the supernatant in aliquots with liquid nitrogen and store at –70 °C.

Large scale (50 L) Hela Cell Nuclear Protein Extraction

Method from Yuan Lab

Note: All steps, buffer, glassware are in cold room or on ice.

1. Estimate the packed cell volume (**PCV**), ~50 ml.
2. Add **5X PCV** (250 ml) of 1X hypotonic Lysis Buffer (final vol. in tube is 300 ml). This is a rapid buffer exchange step. Try to be quickly but gently resuspend the cells.
3. Quickly move them into 50 ml conical tubes and **spin at 3000 rpm for 5 min**.
4. Pour off the sup. Bring the cells to **3X** the original PCV with hypotonic buffer (now the final vol. is 150-200 mls).
5. Let the cell to **swell on ice for 10 min**.
6. Cell disruption: Using a glass tissue homogenizer, **dounce on ice** slowly but steadily **12** times. Check lysis by Trypan Blue solution under the microscope. Lysis should be 80-90%. If the lysis is not sufficient, perform several more strokes until lysis is complete, but avoid dounce too much to break the nuclei.
7. **Centrifuge at 4K for 15 min**.
8. Transfer the supernatant to a fresh tube. This fraction is the *cytoplasmic fraction*.
9. The pellet is nuclei. Estimate the packed nuclear volume (**PNV**, should be bigger than PCV).
10. Add **1/2 PNV** of Low Salt Buffer containing DTT and protease inhibitors to suspend the pellets. Combine and pour into a 500 ml beaker.
11. Add **1/2 PNV** if High Salt Buffer (1.4 M salt) in a slow dropwise fashion while stirring slowly. Note: ad High Salt Buffer drop by drop over 10-15 min period; the stirring should be fast enough to provide good mixing, but not too fast to damage the nuclei. The final salt conc. is 0.35 M.
12. Stir gently for **30 minutes**.
13. Centrifuge for **30 minutes at 12K g (10 K rpm in SS34 rotor)**.
14. The supernatant is nuclear extract. Transfer the supernatant to a clean, chilled tube. Aliquot into 0.5 or 1ml aliquots. Snap-freeze with liquid nitrogen and store at -80 °C.

Solutions for nuclear extraction preparation.

	Final conc.	Stock	add vol. to make solution	
			500 ml	**1 L**
Hypotonic Lysis Buffer	10 mM HEPES7.9	1 M	5 ml	10 ml
	1.5 mM MgCl2	1 M	0.75 ml	1.5 ml
	10 mM KCl	3 M	1.67 ml	3.34 ml
Low Salt Buffer	20 mM HEPES 7.9	1 M	10 ml	20 ml
	1.5 mM MgCl2	1 M	0.75 ml	1.5 ml
	20 mM KCl	3 M	3.34 ml	6.7 ml
	0.2 mM EDTA	0.5 M	0.2 ml	0.4 ml
	25% glycerol		125 ml	250 ml
High Salt Buffer	20 mM HEPES 7.9	1 M	10 ml	20 ml
	1.5 mM MgCl2	1 M	0.75 ml	1.5 ml
	1.4 M KCl	3 M	233.3 ml	466.6 ml
	0.2 mM EDTA	0.5 M	0.2 ml	0.4 ml
	25% glycerol		125 ml	250 ml
			1 L	**4 L**
Dialysis Buffer	20 mM HEPES 7.9	1 M	20 ml	80 ml
	100 mM KCl	3 M	33.3 ml	133 ml
	0.2 mM EDTA	0.5 M	0.4 ml	1.6 ml
	20% glycerol		200 ml	800 ml
Isotonic Cell Lysis Buffer	10 mM Tris 7.5			
	2 mM MgCl2			
	3 mM CaCl2			
	0.3 M sucrose			

Note: add 0.2 mM PMSF (from 100 mM, 500x) and 0.5 mM DTT (from 1 M, 2000x) alonh with PI to all buffers right before use.

3 M KCl 224 g in 1L.
1 M MgCl2 101.7g in 500 ml.