Extraction of cytoplasmic and nuclear proteins

A) Materials:

- Cell (5 to 10 x 10^6 cells)
- PBS
- Nuclear Extract (NE) buffer* (HEPES [20 mM] pH 7.9, NaCl [0.4 M], EDTA [1 mM], Glycerol 25%, Protease Inhibitors 1x (add just before use))
- Cytoplasmic Extract (CE) buffer* (HEPES [10 mM] pH 7.9, KCl [10 mM], EDTA [0.1 mM], NP-40 0.3% (add just before use), protease inhibitors 1x (add just before use))

* for 50 mL of buffers:

<table>
<thead>
<tr>
<th>NE buffer</th>
<th>CE buffer</th>
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<tbody>
<tr>
<td>1 M HEPES</td>
<td>1 M HEPES</td>
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<tr>
<td>5 M NaCl</td>
<td>0.5 mL</td>
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<tr>
<td>0.5 M EDTA</td>
<td>2 M KCl</td>
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<tr>
<td>Glycerol</td>
<td>0.5 M EDTA</td>
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<tr>
<td>Protease</td>
<td>NP-40</td>
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<td>Sufficient for</td>
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B) Procedure:

1. Centrifuge and remove the supernatant from your cell suspension.
2. Add and ressuspend pellet in 1 mL of ice cold PBS.
3. Centrifuge at 6000 rpm for 5 min in cold room.
4. Remove all supernatant.
5. Put tubes on ice and add 5X more CE buffer to your pellet (i.e., if your pellet is 20 \(\mu\)L, add 100 \(\mu\)L of CE buffer).
6. Ressuspend your pellet in this CE buffer and incubate on ice for 5 min. Vortex time to time.
7. Centrifuge in cold room at 3000 rpm for 5 min.
8. Harvest supernatant (this is the cytoplasmic extract. Note that the pellet is not dense).
9. Ressuspend pellet in 100 \(\mu\)L of CE buffer without NP-40.
10. Centrifuge in cold room at 3000 rpm for 5 min. Remove supernatant. If you want to wash better your nuclear pellet, repeat 8 and 9.
11. Add equal volume of NE buffer to this pellet (i.e., if pellet is 40 µL, add 40 µL of NE buffer).

12. Ressuspend and incubate on ice for 10 min. Vortex time to time.

13. Centrifuge at 14,000 rpm for 5 min at 4°C.

14. Harvest supernatant (this is the nuclear extract).

15. Store extracts at -80°C or prepare to WB.

C) Preparing samples to WB:

1. Dilute to 1X the 5X SDS loading buffer in your sample.

2. Boil samples for 5 min at 95 to 100°C.

3. Cool down to RT to load samples or froze them at -20°C.

**SDS loading buffer 5X**

- Tris [1 M] pH 6.8 --------------- 12.5 mL
- SDS -------------------------------- 5 g
  Note: this step denatures proteins and generates a constant anionic charge-to-mass ratio
- Glycerol ------------------------ 25 mL
  Note: this step gives a high density to samples allowing them to sink in the wells
- 2-Mercaptoethanol -------------- 5 mL
  Note: this step reduces disulfide bonds
- Bromophenol Blue --------------- 50 mg
  Note: this step allows low M.W. for dye-front determination
- dd water up to 50 mL