Protein Extraction

Nuclear and Cytoplasmic fractionation from frozen cells

Protocol:

Overview and Considerations:

1. This procedure can be used to fractionate frozen mammalian cells into nuclear and cytoplasmic fraction.

Reagents:

1. Labeled eppendorf tubes (3x the number of samples you have);
2. Ice bucket;
3. Pipets;
4. Microcentrifuge at 4°C;
5. **Low Salt Buffer:** 20mM HEPES pH 7.3, 1mM EDTA, 1mM EGTA, 1mM DTT*, & inhibitors*.
6. **High Salt Buffer:** 20mM HEPES pH 7.3, 1mM EDTA, 1mM EGTA, 420mM NaCl, 20% v/v glycerol, 1mM DTT*, & inhibitors*.
7. **Inhibitors:** 1000X Pic2, 1000X Pic3, 1000X PMSF (100mM), 100X KF (500mM), 100x β-glycerophosphate (500mM), & 1000X PUGNAc (2mM).

* buffers can be made in stock quantities and stored at room temperature. Add DTT (1ug/ml) and inhibitors immediately before use.

Protocol:

1. Determine the volume of Low Salt Buffer and High Salt Buffer you will require. Remember you have numerous wash steps that require 500ul per tube of low salt buffer (1.5mls per sample).
2. Add DTT to the desired volume of buffer.
3. Add inhibitors to the extraction buffer (you do not need to add inhibitors to the low salt buffer that you use in the wash steps). Thus you will have three tubes in your ice bucket: Low salt buffer + DTT; Low salt buffer + DTT + Inhibitors; & High salt buffer + DTT + inhibitors.
4. For 5mls of buffer add:
    - 5ul of 100mM PMSF (Final 0.5mM);
    - 5ul 1000X Pic2;
    - 5ul 1000X Pic3;
    - 5ul 2mM PUGNAc (Final 200nM);
    - 50ul of KF (Final 1mM);
    - 50ul of β-glyerophosphate (Final 1mM).
    - *PMSF is inactivated within 30min. It should be added to buffers immediately before use.*
5. Incubate frozen cell pellets in the desired volume of low salt extraction buffer for 10min on ice. Resuspend cells by gentle pipetting (pipet up and down three times), incubate for an additional 10min.
6. Centrifuge at 4°C for 1min at maximum speed in an eppendorf centrifuge.
    - Transfer the crude cytoplasmic extract to a new tube;
    - Spin the crude cytoplasmic extract at maximum speed in an eppendorf centrifuge for 20min, transfer the supernatant to a new tube;
7. Rinse the pellet with 500ul of low salt wash buffer by gently pipeting the pellet x1. Centrifuge at maximum speed for 10 seconds at 4°C.
8. Aspirate the supernatant;
9. Repeat steps 6 and 7 twice. Pellets will look slightly clearer and smaller. *If the pellet still looks opaque after three washes, perform an additional wash.*

10. Resuspend the pellet in the desired volume of high salt extraction buffer with vigorous pipetting; Incubate on the rocker in the cold room for 30min.
11. Sonicate samples for 5 seconds on setting three (Use the sonicator in the Hart Lab, WBSB 422);
12. Centrifuge samples for 20min at full speed in an eppendorf centrifuge at 4°C;
13. Transfer supernatants to new tubes. Discard pellets.
14. Determine the protein concentration.

Other considerations:

- Fractionations take time, and cells/samples should be kept as cold as possible. If possible, put buffers at 4°C the night before.
- *It is best to determine the protein concentration and make up gel samples before you freeze-samples. The freeze thaw process can induce protein aggregation that can complicate the protein estimation. For samples that have been frozen, respin the samples for 10min to pellet aggregates before performing protein estimations or making up SDS-PAGE samples;.*
- *Samples should be snap frozen on dry-ice;*
- *Samples should be defrosted either on ice or rapidly at 37°C. Do not leave samples at 37°C longer than necessary.*
- *You should blot for Tubulin and Lamin to confirm that you have successfully separated the nuclear and cytoplasmic fractions.*