NE-PER CELL FRACTIONATION PROTOCOL: 6WELL PLATE

Separation of cytosolic and nuclear fractions of cell monolayer

Materials: NE-PER Pierce Kit, HALT, RNase Out Inhibitor, 1.5ml microfuge tubes, cell scrapers, 1x PBS.

Preparation of Reagents:

N.B: Volumes below calculated for 4 wells of a 6-well plate (typical). Recalculate total amount of CER I *cocktail* needed if number of wells differ

CER I Solution:

Add to a 2mL tube the following:

N.B: Create a master mix for all samples, below is enough for one set of 4wells

200 ul CER I
2 ul RNase out Inhibitor (Invitrogen)
2 ul Halt

Protocol:

1. Place cell culture plates on Ice
2. Wash cells with cold 1xPBS
3. Tip plate on side to drain excess PBS
4. Aspirate off
5. Add 1ml of 1x PBS to well and scrape cells.
6. Transfer lysate to next well and scrape
7. Repeat until all 4 wells are scraped
8. Transfer 1.5mL tubes
9. Pellet the cells by centrifuging at 2000 RPM for 5 minutes @ 4°.
10. Aspirate off the PBS. Tip tube upside down to drain PBS while aspirating.
11. Add 204ul of the CERI mixture .
12. Vortex vigorously for 15 seconds
13. Incubate on Ice **10 min MAX (Important!)**
14. Add 11ul cold CERII to each tube
15. Vortex vigorously for 10 sec
16. Incubate on Ice 1 min minimum **(10 min max)**
17. Vortex 10 sec
18. Centrifuge 13,200 rpm for 5 min at 4°
19. Immediately transfer supernatant (Cyto Fraction) to a clean pre-chilled 1.5ml–stay away from pellet
20. Quick spin down pellet and remove excess cyto fraction with an insulin syringe and discard.
21. After all nuclear pellets are collected:

Follow either the NER for Protein isolation ***or*** Trizol for RNA isolation (on next page)

NER: Protein from the nuclear fraction:

1. Add to a new 1.5mL tube (need 1 aliquot/ nuclear sample) and set on ice:
   100 ul NER
   1 ul Halt
   1 ul RNAse OUT
2. Quick spin down pellet (from above) and remove any excess cyto fraction with an insulin syringe and discard
3. Add the NER cocktail to pellet
4. Sonicate 10 Sec (Dr. Chalovich lab)
5. Centrifuge 13,200rpm 10 min at 4°
6. Transfer all Supernatant (nuclear fraction) to clean pre-chilled 1.5ml tube
7. Store -80°C until can be dialyzed (see protocol)

TriZol : RNA Isolation: N.B: Skip NER step

1. Resuspend Nuclear pellet with 700 µl Trizol
2. Triturate suspension with a 3cc syringe/20g needle
3. Store at -80°C Abyss until the Nuclear mRNA is isolated (see TriZol protocol)