NE-PER CELL FRACTIONATION PROTOCOL: 10CM PLATES

Separation of cytosolic and nuclear fractions of 3T3-L1 Preadipocyte cell monolayer
N.B: double reagents for fully differentiated Adipocytes

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

Preparation of Reagents: Calculated for 10cm plate surface area.

CER I Solution:

Add to a 2mL tube the following: (1 tube/6 plates)

N.B: Create a master mix for all samples, below is enough for one set of 6 plates

1200 ul CER I
12 ul RNase out Inhibitor (Invitrogen)
12 ul Halt

Protocol:

1. Place (6) 10cm plates on Ice
2. Wash cells with cold 1xPBS
3. Tip plate on side to drain excess PBS
4. Aspirate off
5. Add 1.5ml of 1x PBS and scrape cells.
6. Transfer lysate to next plate and scrape
7. Repeat until all 6 plates are scraped
8. Transfer the lysate and divide it equally between (2) 1.5mL tubes
9. Pellet the cells by centrifuging at 2000RPM's for 5 minutes @ 4°.
10. Aspirate off the PBS. Tip tube upside down to drain PBS while aspirating.
11. Add 600ul of the CERI mixture to each tube.
12. Vortex vigorously for 15 seconds
13. Incubate on Ice **10 min MAX (Important!)**
14. Add 33ul 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:
   300 ul NER
   3.0 ul Halt
   3.0 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)