

Insoluble/Soluble Fractionation Protocol

V.1 NDJ

5-02-05

Materials:

TST Buffer (see supplementary recipes)

25 gauge needles

1CC syringes

Microfuge tubes and microtube centrifuge

LDS running buffer (Invitrogen)

Sample reducing agent (Invitrogen)

Method:

1. Collect cell culture as in WB protocol with the modifications below.
2. Keep always on ice.
3. Triturate cells 5 x 25gauge needle only.
4. Centrifuge 100ul 15 minutes 13000xg
5. Place supernatant in separate tube
6. Add 50ul 1x LDS running buffer to pellet
7. Pippette and centrifuge pellet to try and get into solution
8. Heat pellet 10 minutes at 70°C
9. Flick and centrifuge pellet briefly.
10. Take 1/4 of each.
 - a. Soluble Fraction
 - i. 25ul of supernatant
 - ii. 3ul reducing agent
 - iii. 8ul 4x LDS buffer
 - b. Insoluble Fraction
 - i. 12.5ul pellet
 - ii. 2ul reducing agent
11. Heat 10 minutes 70°C
12. Load gel—double check order

Assessing inclusion formation by insolubility

