Pulse labeling protocol-04-13-2011

1. wash with 4 ml methionine free DMEM once, prestarve in 4 ml of prestarve media for 10 min. prestarve media: 90% DMEM (methionine free), 10% dialyzed serum. 4 plate, make 4X4+4X1.0=20 ml--25ml, so 22.5 ml of DMEM (methionine free) and 2.5ml of dialyzed serum.
2. Prepare pulse media:

    1.0ml prestarve media with 0.4mci labeling mix per plate

3. Add 1.0 ml pulse media (with 50 uCi of [35S]methionine/ml for 15 min) to each plate in order, 15 mins in incubator.
4. Remove pulse media in order and place in original container.
5. Wash plate with 1X 74ml of cold PBS, snap free in liquid N2.
6. Harvest cells in 300 ul Ab lysis buffer with inhibitors in screw top tubes.
7. Sonicate 12 pulse at output2 , duty cycle 20%.
8. Preclear, add 20ul of protean A beads. 1hour at 4 degree.
9. Spin 14,000x for 10 min. Transfer super to a new tube.
10. Save 30 ul for input, store at -80.
11. Add 2 ul of C33 beads per tube and incubate overnight at 4 degree with rocking in a plastic bag.
12. The next morning, add 20ul of protein A beads for 1 hour
13. Spin at 4500X for 15 seconds. Transfer super to a new tube and save at -80.
14. Wash beads 4x in Ripa buffer with inhibitors with 1 ml each wash.
15. Resuspend beads in 20ul of RIPA buffer plus 10ul of 4XSDS sample buffer. Boil for 10 min and load half on protein gel 9%.
16. Fix gel in methanol:H2O:acetic acid 45:45:10 for 30 min at room temperture with rocking.
17. Gel transfer to membrane
18. Soak in Amplify (Amersham-NAMP100) for 30 min at RT with rocking.
19. Develop membrane.
20. Western blot membrane.