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LARGE-SCALE IMMUNOPRECIPITATION

Guidelines for sample preparation
(How to protect your samples from contamination with keratin)

- **Clean your bench**
- **Try to avoid contact of samples and solutions with dust, skin or hair**
- **Wear gloves at all times**
- **All reagents should be prepared fresh**
- **Use ultra-pure water for all solutions**

1. Harvest cells and wash with cold PBS to remove serum (spin cells at 1500rpm, remove supernatant). Repeat this step once.

2. Resuspend cell pellet in ice-cold Lysis Buffer containing protease inhibitors (1 protease inh. Cocktail tablet (Roche) in 10ml lysis buffer).

3. Vortex and incubate 30 min on ice, or on a rotator/vortex at 4°C

4. Spin at 14,000 rpm at 4°C for 10 min, collect supernatant

5. Determine protein concentration and dilute equal amount of protein with ice-cold NET buffer to protein concentration 1-5 mg/ml (use 15ml Falcon tube if needed)

6. Add 50 µl Protein A agarose (previously washed 3-4x in NET buffer)

7. Incubate on a rotator at 4°C for 60 min

8. Centrifuge at 5000 rpm (for 15ml Falcon tubes: 3000rpm) for 5 min at 4°C, transfer supernatant to a new tube and add 50 µl Protein A agarose conjugated to antibody

9. Incubate on a rotator at 4°C for 2 hours, or overnight

10. Centrifuge at 5000 rpm (for 15ml Falcon tubes: 3000rpm) for 5 min at 4°C

11. Remove supernatant (you can collect it to analyse efficiency of your immunoprecipitation)

12. Add 1-10 ml (depending on a tube size and protein amount) of ice-cold NET buffer and rotate a few times (each sample should be rotated in the same way).

13. Centrifuge at 5000 rpm (for 15ml Falcon tubes: 3000rpm) for 5 min at 4°C

14. Repeat step **12** and **13** three times

15. Transfer to an eppendorf tube, if the immunoprecipitation was carried out in a Falcon tube

16. Centrifuge at 5000 rpm for 5 min at 4°C

17. Elution: add up to 400 µl of ice-cold Elution Buffer, mix and incubate on a rotator at 4°C for 20 min

18. Centrifuge for 5 min at 4°C and collect supernatant

19. Precipitate protein using chloroform-methanol precipitation (see other protocol).

20. Add SDS reducing sample buffer and boil for 3 min at 95°C

Alternatively, elute using techniques performed in the following order:

1. Peptide elution (HA peptide, FLAG peptide, MYC peptide, depending on your tag)

2. Low pH Glycine elution (described above)

3. Boiling beads in SDS reducing sample buffer

REAGENTS

Lysis buffer 1 l
50 mM Tris-HCl pH 7.4 6.05g
0.5% NP-40 5 ml
150 mM NaCl 8.76g
20 mM $MgCl_2$ 4.07g

NET buffer
50 mM Tris pH 7.5
5 mM EDTA
150 mM NaCl
0.5% NP-40

Elution buffer
100mM Glycine pH 2.5