coIP

This is the protocol for 6L of culture at ≈2 OD_{600} and low expressed protein

Everything (Buffers, tubes, incubations…) at 4ºC and washes in the cold room

Buffers and reagents

100mL UBA Buffer (at 4ºC)

60mL	H_2O
2.5mL	HEPES 1M pH7,5
15mL	KoAc 1M
1mL	$MgCl_2$ 1M
0,5mL	$CaCl_2$ 1M
1mL	NP40%
20mL	Glycerol

Magnetic Beads+Ab. For each sample:

1. Wash 37,5 ul Dynabeads Prot G (Invitrogen, 10009D) with 375ul PBS-T0,1% for 4min in the nutator at 4ºC
2. Wash 2 times more
3. Resuspend in 37,5ul of PBS-T0,1% and add the Antibody
   i.e. 1,6ul anti-Flag M2 mouse, Sigma F1804
   i.e. 2,5ul anti-HA rabbit, Sigma H6908
4. Incubate **4-6h** or **O/N** in the wheel
5. Wash 2 times with 375ul PBS-T0,1% for 4min in the nutator at 4ºC
6. Wash 2 times with 375ul UBA buffer for 4min in the nutator at 4ºC
7. Resuspend in 37,5ul UBA buffer

When I beads are concentrated (first, last step) is better to use commercial tips (you loose less, less binding to the wall of the tip)

Magnetic Beads.

Used for cleaning step. For each sample:

1. Wash 100 ul Dynabeads Prot G (Invitrogen, 10009D) with 900ul UBA buffer for 4min in the nutator at 4ºC
2. Wash 2 times more
3. Resuspend in 100ul of UBA buffer

When I beads are concentrated (first, last step) is better to use commercial tips (you loose less, less binding to the wall of the tip)

For negative control of IP do the same, but working with 37,5ul of beads.

Laemmli Buffer 4x (room temperature)

200mM Tris-HCl (pH6,8)
8% SDS
0,4% Bromophenol blue
40% Glycerol
Add DTT before use

DTT 1M

Dissolve 1.5 g of DTT in 8 mL of H2O. Adjust the total volume to 10 mL, dispense into 1-mL aliquots, and store them in the dark (wrapped in aluminum foil) at -20°C

Benzonase and antibodies

Obtaining the lysates for the IP (5-6h)

1. Defrost the sample.
   It takes 1-2h at 4ºC. When it starts melting:
   - Add 1mL UBA Buffer + 1 pill of cOmplete Mini, EDTA-free PI Cocktail
   - Put together the powder for each strain
   - Add UBA Buffer (not much!) and resuspend it (vortex+wheel at 4ºC)
   - Add UBA Buffer up to 32,5mL
2. Add 7,5ul Benzonase (MPI homemade, 3 times more active than Merck one)
3. 1h in the wheel at 4ºC

Cool down centrifuges, rotors and tubes

4. Add UBA buffer up to 50mL and centrifuge max speed for 15min at 4ºC
5. Transfer "SN" to ultracentrifuge cold tubes
6. Ultracentrifugate 40K for 45min at 4ºC with Tfi45.Important to:
   - Use right tubes per your volume, to avoid tube collapse!
   - Weight and adjust volume with UBA buffer to have tubes properly balanced
   - Check that tubes and rotor have all the rubber rings
7. Transfer the supernatant into a new tube with a Pasteur Glass Pipette
8. Add UBA Buffer up to 30-35mL *You can keep at -80ºC*

Immunoprecipitation

9. Add 90ul of washed magnetic beads/sample
10. Incubate 20-30min in the wheel at 4ºC

    ➔ *good moment to wash the magnetic beads for control and the beads+Ab*

11. Put the tubes in the magnetic rack for 1min and transfer SN in a new tube
12. Take some 50-100ul as input
13. Divide SN in 3 tubes (i.e. 10ml/tube) and add the washed beads:
    - Control: 10mL + 36ul washed magnetic beads
    - Ab1: 10mL + 36ul washed magnetic beads+Ab1 (i.e. Flag)
    - Ab2: 10mL + 36ul washed magnetic beads+Ab1 (i.e. HA)
14. Nutate O/N at 4ºC (3h could be also possible)
15. Centrifugate at 700g for 15min at 4ºC
16. Discard most of the SN and transfer the beads into a 1,5mL tube
17. Put the tubes in the magnetic rack for 1min and discard the SN
18. Wash with 500ul UBA Buffer for 4min in the nutator at 4ºC
19. 2 washes with 500ul PBS-T 0,1%
20. Resuspend with 500ul PBS-T 0,1%, transfer into a new tube and wash
21. Resuspend in 40ul of Laemmli Buffer **2x**+0,1M DTT *(better with commercial tips)*
22. Incubate at 95ºC for 5-10min (max shaking)
23. Centrifugate shortly, magnet and transfer in a new tube. *Better with commercial tips*
24. Ready to run or keep at -80ºC. *I use to run 10ul of the eluted and 1-5ul of input. Remember to denature the input at 95ºC for 5-10min (in Laemmli+DTT).*