# Immunoprecipitation

Technique of precipitating a protein antigen out of solution using an antibody that specifically binds to that particular protein. This process can be used to isolate and concentrate a particular protein from a sample containing many thousands of different proteins, to analyze protein-protein interactions, to detect and quantify the presence of two or more known proteins.

## General guidelines

* Antibodies against all protein must be available, if not, use tagged-proteins and overexpression system
* Usual amount of cells/reaction – 1x 10 cm plate, usual amount of protein – 200-500 ug/reaction
* Protocol bellow describes the situation when you work with 10 cm plates and 6-well plates
* For 15 cm plates scale up 3x
* **Work on ice**

## Buffer preparation

* Calculate the amount of lysis buffer you need (1 ml/plate) + approx. 2 ml for equilibrating the beads
* To complete lysis buffer add:
  + **1x protease inhibitors** (get 50x protease inhibitors by dissolving 1 tablet of Roche inhibitors in 1 ml of distilled water, store frozen)
  + **1 mM DTT**
  + Phosphatase inhibitors when phosphorylations need to be preserved (dilute 200x)

optional

* + 0.01 M N-ethylmaleimid (NEM) when ubiquitinations need to be preserved
  + 0.1% SDS to solubilize proteins bound to cytoskeleton

## Procedure

1. Remove medium from the plate by aspiration
2. Wash with 10 ml of PBS, gently stir to wash the remaining medium, aspirate PBS
3. Let the plates stand in the tilted position to collect the rest of PBS
4. Remove the PBS by aspiration

**Note:** Protocol can be interrupted in this stage and "dry" cells on the plate can be frozen at **-80°C**

1. Add 1 ml of lysis buffer and let the lysis continue for **at least** **15 minutes** on ice. Stir gently once in a while
2. Transfer the lysate to eppendorf tube and centrifuge at maximum speed at 4°C for at least 10 minutes.
3. Divide the supernatant into new 1.5 ml eppendorf according to your experimental setup. Standard procedure with two IP antibodies: transfer 400 – 450 µl of cleared lysate into each tube with the antibody and 60 µl to 0.5 ml tube for total cell lysate (TCL)
4. Add 15µl of 5x Laemmli buffer to your TCL samples, store at -20°C until gel run
5. Add 1µg of antibody to each reaction (**Check antibody concentrations** on original vials or in data sheets)
6. Incubate antibody-lysate mixture for 30 minutes on ice
7. Prepare G-protein sepharose beads (approx 15µl solid beads/30µl slurry per reaction)
8. Equilibrate G-protein sepharose beads by washing them twice in complete lysis buffer and centrifuging (**0.1 RCF**, 4°C, 1 minute)
9. Divide the beads into individual samples in larger volume (approx. 40µl/tube) to ensure equal distribution of the beads
10. Place the micro test tubes into the carrousel in the fridge and let them rotate **overnight** (or for at least 4 hours on RT)
11. Take out the micro test tubes from the carrousel, centrifuge (**0.1 RCF**, 4°C, 1 minute)
12. Carefully remove the supernatant and add 800 µl of lysis buffer without protease inhibitors to the beads. Aim to mix the beads by the lysis buffer
13. Centrifuge (**0.1 RCF**, 4°C, 1 minute)
14. Repeat steps 16 & 17 - **5x**
15. Remove the supernatant, add 40 µl of 2x Laemmli buffer (this ensures two WB runs when 20µl are loaded)
16. Boil all the samples for 5 minutes and analyze by WB

## Buffers

**Lysis buffer**

|  |  |  |
| --- | --- | --- |
| reagent | Final concentration | To 250 ml |
| Tris buffer pH 7.4 | 50 mM | 12,5 ml [1M] |
| NaCl | 150 mM | 7,5 ml [5M] |
| EDTA | 1 mM | 0,5 ml [0,5M] |
| NP40 | 0.5 % | 1,25 ml |
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