# IP: Immunoprecipitation Protocol - Standard

In immunoprecipitation (IP) approaches, antibodies are employed to specifically pull down the corresponding target molecule from a complex sample like a tissue or cell lysate. Isolated proteins or complexes can be analyzed further to identify potential interaction partners of the target molecule or to determine the specificity of an antibody.
Tissue or cell extracts have to be solubilized before IP, but you can skip this, if your material is only soluble, e.g. cytosol.

## Materials and reagents

- **Solubilization buffer A:** 10 mM Tris/HCl (pH 7.4), 50 mM NaCl, 2% Triton X-100, protease inhibitors
- **Buffer B:** 10 mM Tris/HCl (pH 7.4), protease inhibitors
- **Buffer C**: 10 mM Tris/HCl (pH 7.4), 150 mM NaCl, 0.2% Triton X-100, protease inhibitors
- **Blocking buffer:** 10 mM Tris/HCl (pH 7.4), 2% BSA, protease inhibitors
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## Procedure

All steps should be carried out at 4°C. Protein solubilization (solubilization step 1) and binding of the antibodies to the beads (immunoprecipitation step 1) can be carried out in parallel.
Protease inhibitors should be included.

### Solubilization with Triton X-100

1. Solubilize proteins by adding a 1:1 volume of **solubilization buffer A** to the cell or tissue sample (final concentration of 5 mM Tris/HCl, 25 mM NaCl, 1% Triton X-100).
   Starting material should have a concentration of 1-4 mg/ml.
2. Centrifuge unsolubilized material at 9600x g for 10 min. The supernatant will be used for IP. If desired, the pellet can be kept for further analysis.

### Immunoprecipitation

1. Incubate 5-10 µg of antibody or 5 µl antiserum with 10 µl Protein G or A slurry in 200 µl **buffer B** for 1 h to bind.
2. Centrifuge beads for 5 min at 2400x g and discard the supernatant.
3. Block beads with 200 µl of **blocking buffer** for 30 min.
4. Centrifuge beads for 5 min at 2400x g and discard the supernatant.
5. Wash beads with **buffer B,** centrifuge beads for 5 min at 2400x g, and carefully remove **buffer B.**
6. Add 100-200 µl of the sample and incubate for 1-2 h at 4°C rotating head over tail.
7. Centrifuge beads for 5 min at 2400x g and collect supernatant for subsequent analysis.
8. Wash suspension twice with **buffer C.** Centrifuge beads for 5 min at 2400x g and remove **buffer C.**
9. For SDS-PAGE analysis, incubate the pellet with SDS loading buffer and apply to SDS-PAGE. Apply starting material and supernatant from step 9 for comparison.

## Remarks

- Some proteins are not efficiently solubilized by Triton X-100. For these proteins, the denaturing solubilization protocol is recommended. For further details have a look at: Geumann C, Grønborg M, Hellwig M, Martens H & Jahn R (2010). A sandwich enzyme-linked immunosorbent assay for the quantification of insoluble membrane and scaffold proteins. Analytical Biochemistry 402: 161-9.
- If membrane proteins are immunoprecipitated, make sure that detergent is included in all steps that contain your target protein.
- Not all IgG subtypes from all species bind equally well to protein A or protein G. It is therefore important to choose the right resin (for details see table 1).

| Species | Subclass | Protein A binding | Protein G binding |
|---|---|---|---|
| Human | IgA | variable | - |
| | IgD | - | - |
| | IgE | - | - |
| | $IgG_1$ | ++++ | ++++ |
| | $IgG_2$ | ++++ | ++++ |
| | $IgG_3$ | - | ++++ |
| | $IgG_4$ | ++++ | ++++ |
| | IgM | variable | - |
| Avian egg yolk | IgY | - | - |
| Cow | IgG | ++ | ++++ |
| Dog | IgG | ++ | + |
| Goat | IgG | - | ++ |
| Guinea pig | IgG | ++++ | ++ |
| Hamster | IgG | + | ++ |
| Horse | IgG | ++ | ++++ |
| Lama | IgG | - | + |
| Monkey (rhesus) | IgG | ++++ | ++++ |
| Mouse | $IgG_1$ | + | ++++ |
| | $IgG_{2a}$ | ++++ | ++++ |
| | $IgG_{2b}$ | +++ | +++ |
| | $IgG_3$ | ++ | +++ |
| | IgM | - | - |
| Pig | IgG | +++ | +++ |
| Rabbit | $IgG_1$ | ++++ | +++ |
| Rat | $IgG_1$ | - | + |
| | $IgG_{2a}$ | - | ++++ |
| | $IgG_{2b}$ | - | ++ |
| | $IgG_3$ | + | ++ |
| Sheep | IgG | +/- | ++ |

**Table 1**