

## IP: Immunoprecipitation Protocol - m6A from Nuclear Extracts

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 molecules or complexes can be analyzed further to identify potential interaction partners of the target molecule or to determine the specificity of an antibody.

### General considerations

Use RNase free molecular biology grade water for all buffers and RNase free tips and tubes.

### Materials and reagents

- **PBS:** Phosphate buffered saline, pH 7.4
- **Protein A or protein G sepharose**
- **Ice**
- **IP buffer:** Tris-HCl pH 7.4, 150 mM NaCl, 0.1% NP40
- **Phenol/chloroform**
- **RNA-elution buffer:** Tris-HCl pH 7.4, 450 mM NaCl, 0.4% SDS
- **96% ethanol**
- **80% ethanol**
- **RNase inhibitor** (e.g. RNasin)

### Procedure

- 10-15 µg antibody per assay are coupled to **protein A or protein G sepharose** in **PBS** at 4°C head-over-tail (several hours).
- The pellet is washed 3 times with ice-cold PBS.
- Incubate immobilized antibody with 20 µl nuclear extract in 250 µl **IP buffer** for 1 h on a head-over-tail rotor at 4°C. The buffer provides stringency to avoid non-specific interaction. Generally, non-specific interactions should be controlled with a parallel pull-down assay using protein A/G sepharose without antibody.
- Wash 5 times with 1 ml of **IP buffer**. After two washes, the content of the reaction tube should be transferred to a new one. This step significantly reduces background in pull-down assays.
- The pellet-bound RNA can be isolated by shaking the tube with 250 µl of **IP buffer** with one volume of **phenol/chloroform** and subsequent ethanol precipitation of the aqueous phase. Alternatively, the precipitated RNA-(complex) may be eluted by shaking with 250 µl of **RNA-elution buffer**. After **phenol/chloroform**-extraction of the eluate protein and RNA-containing phases are precipitated and subjected to analysis.
- RNA-analysis: native RNA may be analyzed by 3'-terminal pCp-labelling or Northern-Blot.

For more background information, refer to [Bringmann P and Luehrmann R, 1987.](#)