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.