

# Protocol for m6A Antibody (Cat. No. 202 003)

## Immunoprecipitation (IP)

### Nuclear Extracts - m6A

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, (200 mM NaCl, 2.5 mM KCl, 8 mM Na<sub>2</sub>HPO<sub>4</sub>, 1.5 mM KH<sub>2</sub>PO<sub>4</sub>), pH 7,4
- **BSA stock-solution:** 20 mg BSA/ml H<sub>2</sub>O
- **Primary antibody:** m6A antibody (cat. no. 202 003)
- **Beads:** Protein A coupled to agarose or sepharose beads
- **Ice**
- **IP buffer:** (10 mM Tris, 150 mM NaCl, 0.1% NP40), pH 7,4
- **Blocking buffer:** IP buffer supplemented with BSA (0,5 mg/ml)
- **Phenol/chloroform**
- **RNA-elution buffer:** (10 mM Tris-HCl, 450 mM NaCl, 0.4% SDS). pH 7,4
- **96% ethanol**
- **80% ethanol**
- **RNase inhibitor** (e.g. RNasin)

### Procedure

1. Wash 30 µl of **beads** in 0,5 ml of **IP buffer** supplemented with **RNasin** and centrifuge beads for 5 min at 2400x g and discard the supernatant.
2. Resuspend the **beads** in 0,5 ml of **blocking buffer** supplemented with **RNasin** and incubate on a rotating wheel for 1 h at 4°C. Centrifuge beads for 5 min at 2400g, remove and discard the supernatant
3. Wash **beads** twice in 0,5 ml of **IP buffer** supplemented with **RNasin**. After each washing steps centrifuge beads for 5 min at 2400g.
4. Equally divide **beads** between two microcentrifuge tubes (one for the IP sample and one for the bead-only control).
5. Incubate 10-15 µg antibody per assay with the prepared **beads** in **PBS** on a rotating wheel for 1 h at 4°C.
6. Wash **beads** with **IP buffer**, centrifuge **beads** for 5 min at 2400x g, and carefully remove IP-buffer.
7. 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.

8. Wash **beads** 5 times with 1 ml of **IP buffer**. After each washing step, centrifuge **beads** for 5 min at 2400x g and discard the supernatant. After two washes, the content of the reaction tube should be transferred to a fresh tube. This step significantly reduces background in pull-down assays.
9. 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.
10. 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.](#)