SYSY Antibodies

Tailor-made Antibodies
and Tools for Life Science

Protocol for m6A Antibody (Cat. No. 202 011)
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_2HPO_4, 1.5 mM KH_2PO_4), pH 7,4
- **BSA stock-solution:** 20 mg BSA/ml H_2O
- **Primary antibody:**m6A antibody (cat. no. 202 011)
- **Beads:** Protein G 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.