Protocol for m3G-cap, m7G-cap Antibody (Cat. No. 201 011)
Immunoprecipitation (IP)
Nuclear Extracts - m3G

In immunoprecipitation 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
- **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, 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** twice in 0,5 ml of **IP buffer** supplemented with **RNasin** and **c**entrifuge 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. 10 - 20 µg antibody per assay are coupled to **beads** in **PBS** at 4°C rotating head over tail for 1h.

6. The pellet is washed with **IP-buffer**. Centrifuge **beads** for 5 min at 2400x g after washing discard the supernatant.

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. Generally, non-specific interactions should be controlled with a parallel pull-down assay using protein A/G-sepharose without antibody.

8. Wash five 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 Bochnig P et al., 1987.