# 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.*