RNA immunoprecipitation (RIP)

Use 5-10x10^6 cells per RIP.

- Wash cells twice in cold PBS, harvest and pellet cells by centrifugation (3 min 4°C, 3.000 rpm)
- Suspend cells in 1ml IP Buffer, rotate lysate for 1 h at 4°C
- Centrifuge for 15 min at 12.000 rpm (4°C),
- Transfer supernatant (= cleared lysate) to a new tube, keep 100 µl lysate as input
- Add 10 µl bead-bound anti-GFP antibody (GFP-Trap from ChromoTek) to 900 µl lysate, rotate for 3 h at 4°C
- Wash beads three times with 1 ml IP Buffer
- Resuspend beads in 200 µl IP Buffer,
- Take 40 µl (20%) to monitor immunoprecipitated proteins on western blots
- Spin down residual beads
- Add 40 µg Proteinase K in 100 µl Proteinase K buffer and digest for 30 min at 52°C while shaking
- Spin down beads and transfer the supernatant (= eluted RNA) to a new tube
- Add 1 ml TRI Reagent (Sigma, cat. no. 93289) to the supernatant, the beads and the input lysate, isolate RNA
- Add 20 µg glycogen before RNA precipitation as a carrier
- Analyse RNA by RT-PCR

IP Buffer

20 mM Tris-HCl (pH 8.0)
200 mM NaCl
1 mM EDTA
1 mM EGTA
0.5% Triton X-100
1x complete protease inhibitors (Roche, cat. no. 04693132001)
100 U/ml RNAsin RNase inhibitors (Promega, cat. no. N2511)

Proteinase K Buffer

10mM Tris HCl (pH 8.0)
50 mM NaCl
5 mM EDTA
0.5% SDS

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