Immunoprecipitation Protocol
- using protag-HiPur Agarose Beads -

Solutions and reagents

Lysis buffer [(1)]
Washing buffer [(1)]
Tris-buffered saline (TBS) pH 7.4
2x SDS sample buffer

Remarks

[(1)] protag-HiPur Agarose Beads are compatible with most common lysis/washing buffers (e.g. RIPA). For custom buffers please refer to the buffer compatibility information on the product datasheet.

Procedure

1. Prepare native cell lysates (0.2 to 1.5 ml volume) according to established protocols.
   For mammalian cells, we recommend using 10^6-10^8 cells per experiment.
2. Clarify lysate by centrifugation for 10 min at > 14000 x g and 4 °C.
   Take sample for further analysis (input fraction).
3. Equilibrate protag-HiPur Agarose Beads
   a. Resuspend protag-HiPur Agarose Beads.
   b. Transfer 20 µl slurry (10 µl packed beads) into a clean 1.5 ml reaction tube.
   c. Add 1 ml lysis buffer.
   d. Centrifuge for 1 min at 1000 x g and carefully remove supernatant.
   e. Repeat steps c-d once.
4. Add clarified lysate from step 2 to equilibrated protag-HiPur Agarose Beads obtained in step 3.
5. Incubate 1 h at 4 °C with head-over-tail rotation.
6. Sediment beads by centrifugation for 1 min at 1000 x g and 4 °C.
   Take sample from supernatant for further analysis (non-bound fraction).

Proceed using either the Mini Spin Columns protocol or the batch protocol. (page2)

Progen Biotechnik GmbH • Maaßstraße 30 • 69123 Heidelberg, Germany
Telefon: +49 6221 8278-0 • Fax: +49 6221 8278-24 • E-Mail: info@progen.de • Web: www.progen.com
Version: 1 Date of release: 23.01.2020

Mini Spin Columns (not included)

7. Washing
    a. Carefully remove supernatant.
    b. Resuspend beads in 1 ml lysis buffer
    c. Centrifuge for 1 min at 1000 x g.
    d. Remove supernatant.
8. Transfer
    a. Remove bottom plug from Mini Spin Column. Place column in 2 ml reaction tube.
    b. Resuspend beads in 200 µl lysis buffer, transfer suspension to Mini Spin Column.
    c. Wash out beads sticking to tube with 200 µl lysis buffer and transfer to column.
    d. Centrifuge column for 1 min at 1000 x g, discard flow-through.
9. Wash twice with 400 µl washing buffer, centrifuge for 1 min at 1000 x g.
10. Wash once with 400 µl TBS, centrifuge for 1 min at 3000 x g.
11. Attach bottom plug and place Mini Spin Column in a clean 1.5 ml reaction tube.
12. Resuspend protag-HiPur Agarose Beads in 50 µl 2x SDS sample buffer.
13. Heat Mini Spin Column to 95 °C for 2 min.
14. Remove bottom plug and centrifuge for 1 min at 3000 x g. Boil collected eluate for 5 min at 95 °C and analyze by SDS-PAGE.

Batch Protocol

Due to the more effective washing steps, we recommend using Mini Spin Columns for immunoprecipitation experiments.

7. Washing
    a. Carefully remove supernatant.
    b. Resuspend beads in 1 ml lysis buffer.
    c. Centrifuge for 1 min at 1000 x g.
    d. Wash beads 2-3 times with washing buffer.
    e. Wash beads once with TBS.
8. Transfer beads in clean 1.5 ml reaction tube.
9. Centrifuge for 1 min at 3000 x g.
10. Carefully and completely remove supernatant.
11. Resuspend protag-HiPur Agarose Beads in 50 µl 2x SDS sample buffer.
12. Heat for 5 min to 95 °C.
13. Centrifuge for 1 min at 3000 x g.
14. Collect supernatant and analyze by SDS-PAGE.

Progen Biotechnik GmbH • Maaßstraße 30 • 69123 Heidelberg, Germany.
Telefon: +49 6221 8278-0 • Fax: +49 6221 8278-24 • E-Mail: info@progen.de • Web: www.progen.com
Version: 1 Date of release: 23.01.2020