Immunoprecipitation with SureBeads™ Protein A/G Magnetic Beads

Protocol

IP

For use with immunoprecipitation (IP) tested monoclonal and polyclonal antibodies
This method provides a general procedure for use with the majority of Bio-Rad reagents. In some cases specific recommendations are provided on product datasheets, and these methods should always be used in conjunction with product and batch specific information provided with each vial. Please note that a certain level of technical skill and immunological knowledge is required for the successful design and implementation of these techniques - these are guidelines only and may need to be adjusted for particular applications.

Reagents

4x Laemmli Sample Buffer
β-mercaptoethanol
ddH_2O
Immunoprecipitation tested antibody
(as indicated on the datasheet)
PBS-T (Phosphate Buffered Saline (PBS) + 0.1% Tween®-20)
SureBeads Protein A or Protein G Magnetic Beads

Method

Pre-clearing of the lysate (optional step)

1. Take 100 µl of lysate and add antibody of the same species and isotype as the antibody to be used for the immunoprecipitation procedure at a 1/20 dilution. Incubate on ice for 1 hr.
2. Thoroughly resuspend the SureBeads Magnetic Beads in their solution and transfer 100 µl (1 mg at 10 mg/ml) of SureBeads to a 1.5 ml tube. Magnetize beads and discard supernatant.
3. Wash with 1 ml PBS-T
   a. Resuspend the beads thoroughly in PBS-T.
   b. Magnetize beads and discard supernatant, repeat three times (3x).
4. Add the (pre-cleared) lysate to the washed beads and incubate for 30 mins at 4°C on a rotator. Spin at 2500 rpm for 1 min. Magnetize and retain the supernatant for IP. Discard beads.

Immunoprecipitation

1. Thoroughly resuspend the SureBeads Magnetic Beads in their solution and transfer 100 µl (1 mg at 10 mg/ml) of SureBeads to a 1.5 ml tube. Magnetize beads and discard supernatant.
2. Wash with 1 ml PBS-T
   a. Resuspend the beads thoroughly in PBS-T.
   b. Magnetize beads and discard supernatant, repeat 3x.
3. Add 1–10 µg of antibody in a final volume of 200 µl and resuspend the beads.
4. Rotate for 10 min at room temperature (RT).
5. Magnetize beads and discard supernatant.
6. Wash with 1 ml PBS-T:
   a. Resuspend the beads thoroughly in PBS-T.
   b. Magnetize beads and discard supernatant, repeat 3x.
7. Add the pre-cleared lysate to the beads and rotate for 1 hr at RT.
8. Magnetize beads and discard supernatant.
9. Wash with 1 ml PBS-T:
   a. Resuspend the beads thoroughly.
   b. Magnetize beads and discard supernatant, repeat 3x. Before the last magnetization, transfer the resuspended beads to a new tube. Magnetize and remove supernatant.
10. Spin all tubes down for several seconds at 2500 rpm.
11. Magnetize beads and aspirate off residual buffer.
12. Prepare 50 µl of 1x reduced Laemmli Sample Buffer by mixing 11.25 µl 4x Laemmli Sample Buffer with 1.25 µl β-mercaptoethanol and 37.5 µl ddH_2O.
13. Add 40 µl 1x reduced Laemmli Sample Buffer and incubate for 10 min at 70°C*.
14. Magnetize beads and move eluent to a new vial. At this stage the sample may be stored at -20°C.
15. Boil for 5 mins at 95°C prior to loading onto a SDS-PAGE gel or performing western blotting analysis.

Notes

- Whether to use Protein A or Protein G Magnetic Beads depends on the host species and isotype of the primary antibody to be conjugated to the beads. For information about Protein A and G binding affinities visit: **bio-rad-antibodies.com/ip**
- Appropriate controls should always be carried out. It may be useful to include a mock-IP sample in which the IP is carried out with an antibody of the same isotype as the actual IP antibody.

* Depending on your experimental requirements, you may want to perform an elution step in sample buffer containing no reducing agents. In this type of protocol the reducing agent is added to the supernatant after the heat denaturing step.

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