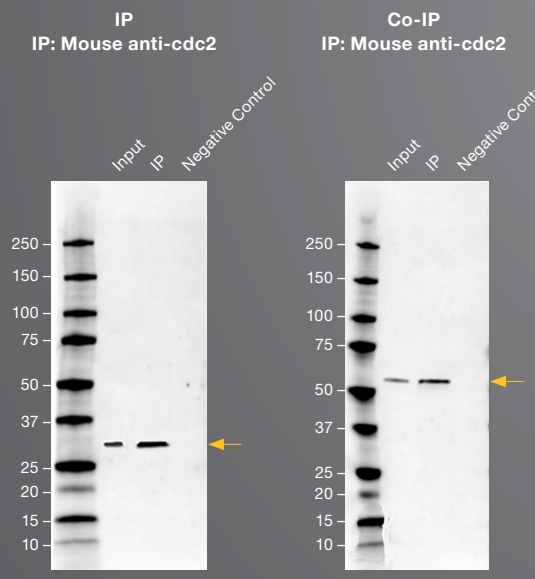
Six Tips to Improve Your Co-IP Results

Achieve Successful Co-IP with SureBeads Magnetic Beads

Co-immunoprecipitation (Co-IP) is a good tool for identifying target protein complexes and interacting partners. Though highly informative, Co-IP is also a challenging technique to master. Below are real results from our Co-IP experiments with SureBeads Magnetic Beads.

We've included six useful tips and a protocol to help you see the same success.



Blot: Rabbit anti-cdc2 (34 kD)

Blot: Rabbit anti-Cyclin B1 (55 kD)

Successful IP and Co-IP results. HEK293 whole cell lysates were immunoprecipitated with cdc2 using the SureBeads IP protocol. Cyclin B1 was successfully co-immunoprecipitated with cdc2.

Tips

1. Samples

- Select biologically relevant samples that have your target protein complex
- Avoid high concentrations, harsh detergents like SDS, and additives that will cause protein complex dissociation

2. Immunoprecipitation

- Maintain protein complexes by using freshly prepared lysates
- Select capture reagent based on the host species of the capture antibody. Protein A has higher affinity for rabbit IgGs. Protein G has higher affinity for mouse IgGs

3. Unidirectional Co-IP

- Due to differences in abundance, you may observe Co-IP in one direction, rather than two. When this occurs, immunoprecipitate the component that has the highest percentage of its total population bound to its partner

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Tips

4. Other Antibodies

- Try another antibody if you do not observe Co-IP. Only unbound protein will immunoprecipitate if the epitope of the capture antibody overlaps the protein-protein interaction site

5. Positive and Negative Controls

- To show that the IP worked, include a blot for just the immunoprecipitated component (positive control)
- To show that the Co-IP worked, include a blot for just the SureBeads Magnetic Beads or IgGs (negative control)

6. Analysis

- To avoid antibody detection in the western blot, use a detection antibody from a different host species than the antibody used in the IP

SureBeads Magnetic Rack and SureBeads Magnetic Beads.

Ordering Information

Catalog #	Description
1614013	**SureBeads Protein A Magnetic Beads**, 3 ml
1614023	**SureBeads Protein G Magnetic Beads**, 3 ml
1614916	**SureBeads Magnetic Rack**, 16-tube holder
1614011	**SureBeads Protein A Magnetic Beads**, 1 ml
1614021	**SureBeads Protein G Magnetic Beads**, 1 ml
1614833	**SureBeads Starter Kit Protein A and G**, Protein A and G beads and magnetic rack
1614813	**SureBeads Starter Kit Protein A**, two Protein A beads and magnetic rack
1614823	**SureBeads Starter Kit Protein G**, two Protein G beads and magnetic rack

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Co-IP with SureBeads Magnetic Beads Protocol

Sample Preparation

1. Determine the best cell line for target of interest.
2. Grow at least 3.6 x 10^7 cells (two 80–95% confluent T-175 flasks).
3. Split cultures 24–48 hours prior to harvest.
4. Grow cells to mid-log growth (determine corresponding confluency or density).
5. Lyse cells using this lysis buffer:
   - 20 mM Tris (pH 7.5)
   - 150 mM NaCl
   - 1 mM EDTA
   - 1 mM EGTA
   - 1% Triton X-100
   - Add fresh protease inhibitor before use
6. IP freshly prepared lysates to avoid complex dissociation.
7. IP using SureBeads Magnetic Beads.

Co-Immunoprecipitation

1. Select SureBeads Protein A or Protein G Magnetic Beads appropriate for the antibody used for IP.
2. Vortex SureBeads to resuspend them and transfer 100 µl (1 mg at 10 mg/ml) of SureBeads to tube.
3. Magnetize beads and discard supernatant.
4. Wash 3x with 1 ml PBS-T (1x PBS + 0.1% Tween-20).
5. Add 7.5 µg of antibody. Rotate 20 min at room temperature.
6. Wash 3x with 1 ml PBS-T.
7. Add 1 mg of antigen-containing lysate. Rotate 1 hr at room temperature.
8. Magnetize beads and wash 3x with 1 ml PBS-T.
9. Elute with 20 µl of 2x Laemmli Buffer. Incubate for 5 min at 90°C.
10. Magnetize and transfer supernatant to new vial (IP sample).
11. Elute again with 20 µl of 2x Laemmli Buffer. Incubate for 5 min at 90°C.
12. Magnetize and transfer supernatant to new vial (IP sample).
13. Run SDS-PAGE.

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