Immunoprecipitation Kit

Product information for BS688/BS689/BS690:

Introductions:

Immunoprecipitation (IP) can specifically purify a protein from a complex mixture of proteins using a specific antibody and a matrix that binds the antibody. The matrix bound protein (via the specific antibody) can then be separated from the mixture by centrifugation. The matrices commonly used are agarose or sepharose bound Protein A or protein A/G PlUS. IP followed by SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE) and immunoblotting is routinely used in a variety of applications: to measure the molecular weights of protein antigens, study protein/protein interactions, determine specific enzymatic activity, monitor protein post-translational modifications and determine the presence and quantity of proteins. The IP technique also enables the detection of rare proteins, which otherwise would be difficult to detect, since immunoprecipitation can concentrate them as much as 10,000-fold.

Component:

BS688-A: Protein A-Agarose (20 reactions)

BS689-A/G Plus: Protein A/G PLUS-Agarose (20 reactions)

BS690: Protein G- Agarose (20 reactions)

Component	BS688	BS689	BS690
10x Lysis buffer	10 ml	10 ml	10 ml
PMSF	0.6 ml	0.6 ml	0.6 ml
10x IP	50 ml	50 ml	50 ml
SDS(10%)	5 ml	5 ml	5 ml
NaCl(5M)	10 ml	10 ml	10 ml
Protein A -Agarose	0.4 ml	/	/
Protein A/G PLUS-Agarose	/	0.4 ml	/
Protein G- Agarose	/	/	0.4 ml
20x PBS	20 ml	20 ml	20 ml
1x loading buffer	1 ml	1ml	1 ml
Spin columns, tubes, caps	20	20	20

Storage :

Protein A/G-Agarose, Protein G- Agarose and Protein A-Agarose should be stored at 4℃; PMSFand1x loading buffer should be stored at-20℃; Others can be stored at RT.

Purified antibody is required but not included as part of kit:

Procedures:

1. Preparation: add 18ul per reaction of Protein A-Agarose (or 18ul Protein-A/G PLUS-Agarose or 18ul Protein G- Agarose)to spin column(containing filter to hold agarose), break off the cap from the column.(Do not discard the cap. The cap will serve in stage 10.), add 700ul PBS to wash agarose, spin; wash four times total. The Protein A-Agarose is now ready to use.
2. Collect cell from 5×10^{6}～1×10^{7} cell culture by centrifuging at 800 rmp for 3 minutes at 4°C. Discard media and wash cell pellet with pre-cold PBS for two times. Spin at 800rpm for 3 minutes during each wash. Drain completely to remove PBS and keep cell pellets. add 1ml 1x Lysis buffer into 100mg cell pellet, vortex,homogenize them with glass homogenizer for 30-50 strokes or sonicated them for 30 seconds,interval 1minutes,repeat operation for three times.centrifuge and collect supernatan as cell lysate.
3. Add 0.7ml cell lysate, add 7ul PMSF, and 1ug purified antibody into a new microcentrifuge,
4. Incubate for 1 hr to overnight at 4^{0} C on flat platform.
5. After incubation, transfer the cell lysate to the washed Protein A beads in the spin column.
6. Incubate for 2 hrs to overnight at 4^{0} C on flat platform.
7. Insert each column into the 2 ml microcentrifuge tubes supplied.
8. Spin at 12,000 x g for 30 seconds at 4^{0} C.
9. Wash the beads in the spin column in 700 ul of 1 X IP buffer six times; wash the beads with 0.1 X IP buffer. Centrifuge the spin column at 12,000 x g for 30 seconds.
10. Add 40-50 ul of 1X Loading buffer on the beads, mix the beads gently (no vortexing).
11. Close the spin column tightly with the cap.
12. Heat samples at 95^{0} C for 5 minutes; adsorb the water around the cap with tissue paper or filter paper.
13. Open the column cap, insert the column back into a new microcentrifuge tube, centrifuge at 12,000 x g for 30 seconds. The eluted immunoprecipitate is ready for SDS-PAGE.

Notes:

1. 1X cell lysis buffer can be used in most cell lysis process (Add SDS if needed in 1:200,).
2. It is important to add protease inhibitors(such as PMSF, aprotinin) to the cell lysis solution.
3. The number and type of washes are important when dealing with non-specific binding.
4. When exhaustive removal of SDS is required, 0.1X IP buffer can be replaced by PBS.
5. To reduce background caused by non-specific adsorption of irrelevant cellular proteins to the Protein A-Agarose, a pre-clearing step can sometimes be useful:

a) Add 18ul of the Protein A-Agarose suspension(add 1ug of control IgG if possible) and to the cell extract sample in a microcentrifuge tube, and incubate for 1hr at 4℃ in rocking platform.

b) Pellet the beads by centrifugation at 12,000x g for 30 seconds and collect the supernatant to a fresh

tube. The sample is now ready for immunoprecipitation as described.

6.When non-specific background binding of proteins is observed, it may be necessary to modify the washing protocol and increase the stringency of the washing conditions by adding salt and/or SDS as follows.

	Option 1	Option 2	Option 3
First 2 Washes	1x IP Buffer/ 0.5M NaCl	1x IP Buffer/ 0.5M NaCl/ 0.1% SDS	1x IP Buffer/ 0.1%SDS
Next 4 washes	1x IP Buffer	1x IP Buffer	1x IP Buffer
Last 1 washes	0.1x IP Buffer or PBS	0.1x IP Buffer or PBS	0.1x IP Buffer or PBS

7.In cases where Protein-A does not bind the specific antibody efficiently, use Protein- A/G Plus or adding a bridging antibody between the specific antibody and the Protein-A may be helpful.

a) Wash the Protein A-Agarose beads twice with PBS.

b) Add 5 to 10 ul of the bridging antibody and incubate for 30 minutes to 1 hour at room temperature. Wash twice with PBS. The Protein-A complex is ready to be use.

Troubleshootings:

1. **If no signals or signals are weak; check the following**:

a. To reduce risk of antigen degradation during sample preparation, add protease inhibitors.

b. Increase the concentration of the primary antibody and prolong the incubation time.

c. Shorten the washing times; use washing buffers with lower stringency (no detergent).

d. Prolong the incubation time with protein A-agarose (overnight).

e. Check the affinity of the primary antibody to protein A-agarose if the binding affinity is low, change to an appropriate matrix.

f. Increase the amount of protein applied to the gel.

g. Check for efficient blotting. Change the transfer condition, buffer etc.

h. Check the detection system or prolong the detection time.

2. If background is too high, check the following:

a: Increase rounds of pre-absorption to remove all proteins binding non-specifically to protein A;

b: Increase the washing time of the antibody-Protein A-Agarose complex or increase the stringency of washing conditions.

c: Avoid touching the membranes; Use clean equipment, fresh buffers and new membranes.

d: Dilute the protein concentration in the sample; decrease the antibody concentrations.

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