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Product Information

Protein G Immunoprecipitation Kit

Catalog Number **IP50**
Storage Temperature 2–8 °C. Do Not Freeze

TECHNICAL BULLETIN

Product Description

The Protein G Immunoprecipitation Kit is especially designed to allow maximal recovery of immuno-precipitates. The whole process is performed in mini-spin columns, instead of in microcentrifuge tubes, which enables convenient washing of the antigen-antibody-bound beads.

The kit has several advantages:

- Minimal loss of antigen-antibody bound beads during washing
- Minimal or no non-specific signals by increasing the stringency of the washing steps

Immunoprecipitation (IP) is a method by which a protein can be specifically purified 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 bound Protein A, G, or L, or Anti-Ig bound to agarose. IP is one of the most widely used immunochemical techniques. IP followed by 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.

Components

Sufficient for performing 50 IP assays.

- 10× IP Buffer, Cat.No.I5779 40 ml
- 5 M NaCl, Ca. No. S6546 15 ml
- 10% SDS, Cat. No. 71736 1 ml
- Protein G Agarose, Cat. No. P3296 2.0 ml
- Spin columns and caps, Cat. No.S3563 50 each
- Microcentrifuge tubes, 2 ml, Cat.No. T7813 50 each

Reagents and equipment required but not provided

- Microcentrifuge tubes
- Protease inhibitor cocktails:
  Cat. No. P2714 – for general use, includes EDTA
  Cat. No. P8340 - for use with mammalian cell and tissue extracts
  Cat. No. P8465 - for use with bacterial cell extracts, includes EDTA
  Cat. No. P8215 - for use with fungal and yeast extracts
- 2× Laemelli Sample Buffer, Cat No. S3401
- Optional: PBS, Cat. No. P3813,
  or TBS, Cat. No. T5030

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store all buffers and reagents at 2–8 °C. To assure preservation of the 10× IP Buffer, it is recommended to open it under sterile conditions. Store columns and tubes at room temperature.

Preparation Instructions

It is recommended to use ultrapure (17 MΩ·cm or equivalent) water when preparing the reagents.

- 1× IP Buffer - For ten IP reactions, dilute 6.5 ml of 10× IP buffer with 58.5 ml of ultrapure water to make 65 ml of solution. The diluted solution is stable for at least 4 weeks when stored at –20 °C.
- 0.1× IP Buffer - For ten IP reactions, dilute 0.7 ml of the 1× IP buffer with 6.3 ml of ultrapure water to make 7 ml of solution.
- 1× IP Buffer with 0.5 M NaCl - For ten IP reactions, mix 1.05 ml of 5 M NaCl, 1.5 ml of 10× IP Buffer, and 12.45 ml of ultrapure water to make 15 ml of solution. The diluted solution is stable for at least 4 weeks when stored at –20 °C.

- 1× IP buffer with 0.1% SDS - For ten IP reactions, dilute 0.15 ml of 10% SDS with 15 ml of 1× IP Buffer to make 15 ml of solution. The diluted solution is stable for at least 4 weeks when stored at –20 °C.
- 1× IP buffer with 0.1% SDS and 0.5 M NaCl - For ten IP reactions, mix 0.15 ml of 10% SDS, 1.05 ml of 5 M NaCl, 1.5 ml of 10× IP Buffer, and 12.3 ml of ultrapure water to make 15 ml of solution.
- Protein G Agarose - Just before use, wash 30 µl of Protein G Agarose per each IP reaction, with 1 ml of cold 1× IP Buffer or PBS by gently vortexing and spinning in a microcentrifuge at 12,000 × *g* for 30 seconds. Remove the supernatant carefully by aspiration. Repeat twice. Add 50 µl of fresh 1× IP Buffer or PBS. The beads are ready to be used.

Procedure

Notes:

- Cell lysis is an important step when performing immunoprecipitation. The detergent of choice should solubilize the antigen of interest without denaturing it or altering its immunoreactivity. The 1× IP Buffer can be used in most cases. Nevertheless, it should be individually checked in cases where enzymatic activity or weak protein:protein interactions are studied.
- It is critical to add protease inhibitors to the cell lysis solution (see Reagents and Equipment Required but Not Provided).
- The number and type of washes are important when dealing with non-specific binding. The Basic Protocol is a suggested guideline. When undesired background signals are observed use the Support Protocol and Supplementary Information for troubleshooting.

A. Basic Protocol

1. Add the following components into an empty spin column:

Component	Amount	Comments
Cell Lysate	100-600 µl	• Amount depends on protein abundance • Add protease inhibitors
Specific antibody	Antiserum 0.5-5 µl Culture supernatant 5-100 µl Ascites fluid 1-5 µl Purified antibody 1-5 µg	Titration is recommended: Add a variable amount of antibody to a constant amount of cell lysate
1× IP Buffer	Complete to final volume of 600 µl	

2. Cap the spin column and incubate for 1 hour to overnight at 2–8 °C, mixing the sample by inversion.
3. During incubation, wash 30 µL per reaction of Protein G Agarose beads (see Preparation Instructions).
4. After the 1 hour incubation, transfer the washed Protein G beads to the cell lysate in the spin column. If beads remain in their washing tube they may be collected by adding 20–30 µl of 1× IP Buffer.
5. Incubate for 2 hours to overnight at 2–8 °C, mixing the sample head-over-tail.
6. Break off the tip of the spin column (Figure 1 B). Do not discard the tip. The inverted tip serves in later steps as a closure to the column (step 14, see Figure 1 C).
7. Insert each column into one of the 2 ml microcentrifuge tubes supplied.
8. Spin in a microcentrifuge at 12,000 × *g* for 15–30 seconds at 2–8 °C. Discard the effluent.
9. Wash the beads in the spin column by resuspending in 700 µl of 1× IP Buffer.
10. Centrifuge at 12,000 × *g* for 15–30 seconds at 2–8 °C. Discard the effluent.
11. Repeat steps 9 and 10 five more times.
12. Perform the last wash with 0.1× IP Buffer.

Note: When exhaustive removal of detergent is required, 0.1× IP Buffer can be replaced by PBS or TBS in the last wash.

The sample is now ready for protein activity assays, which can be performed in the spin column, or for other downstream applications, e.g., analysis by SDS-PAGE.

Sample preparation for SDS-PAGE

13. Centrifuge the spin column at 12,000 × *g* for 15–30 seconds at 2–8 °C.
14. Close the spin column tightly with the inverted tip (see Figure 1 C).
15. Add 40–100 µl of 1× Laemelli sample buffer on the beads.
16. Mix the beads gently (no vortexing) in order to avoid spreading the beads on the column walls.
17. Insert the closed column into a new microcentrifuge tube and transfer to a heating block preheated to 95 °C.
18. Heat samples for 5 minutes. Be sure the Protein G Agarose complex is within the heating well.
19. Open the column tip, insert the column back into the microcentrifuge tube, and centrifuge at 12,000 × *g* for 30 seconds.

The eluted immunoprecipitate is ready to be loaded on a SDS-PAGE gel.

B. Support Protocol

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.

The following table describes options of increasing washing stringency, which are to be used in steps 9 to 11 of the Basic Protocol.

	Option 1	Option 2	Option 3
First 2 washes	1× IP Buffer with 0.5 M NaCl	1× IP Buffer/ 0.5 M NaCl/ 0.1% SDS	1× IP Buffer/ 0.1% SDS
Next 4 washes	1× IP Buffer	1× IP Buffer	1× IP Buffer
Last wash	0.1× IP Buffer, PBS, or TBS	0.1× IP Buffer, PBS, or TBS	0.1× IP Buffer, PBS, or TBS

Note: When exhaustive removal of detergent is required, 0.1× IP Buffer in the last wash can be replaced by PBS or TBS.

C. Supplementary Information

1. Preclearance step
   To reduce background caused by non-specific adsorption of irrelevant cellular proteins to the Protein G Agarose, a pre-clearing step can sometimes be useful:

   a. Add 30 µl of the Protein G Agarose suspension to the cell extract sample in a microcentrifuge tube and incubate for 3 hours to overnight at 2–8 °C in a suitable shaker or rocking platform.
   b. Pellet the beads by centrifugation at 12,000 × *g* for 30 seconds in a microcentrifuge and collect the supernatant (precleared cell lysate) to a fresh tube. The sample is now ready for immunoprecipitation as described.

2. Bridging Antibody
   In cases where Protein G does not bind the specific antibody efficiently, adding a bridging antibody between the specific antibody and the Protein G may be helpful. The bridging antibody is an anti-Ig specific for the species/isotype of the immunoprecipitating specific antibody, which is made in a species that binds well to Protein G.

   a. Wash the Protein G Agarose beads twice with PBS.
   b. Add 5–10 µl of the bridging antibody and incubate for 30 minutes to 1 hour at room temperature
   c. Wash twice with PBS.
   d. The Protein G complex is ready to be added in step 4 of the Basic Protocol.

3. Binding characteristics of Protein G Agarose
   The affinities of immunoglobulins for Protein G vary between different species. Although Protein G is recommended for most species, it is not recommended for detection of mouse IgA, IgM, or IgD.

Protein G binding characteristics:

Species	Immunoglobulin	Protein G Binding
Mouse	IgG1	++++
	IgG2a	++++
	IgG2b	+++
	IgG3	+++
Rat	IgG1	+
	IgG2a	++++
	IgG2b	++
	IgG2c	++
Human	IgG1	++++
	IgG2	++++
	IgG3	++++
	IgG4	++++
	IgM	-
	IgA	-
	IgE	-
	IgD	-
	Fab	++
	K light chains	-
	L light chains	-
	scFv	-
Rabbit		+++
Sheep		++
Goat		++
Horse		++++
Guinea pig		++

References

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2. Antibodies: A Laboratory Manual, Eds., Harlow, E., and Lane, D., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1988).
3. Meier, T., *Anal. Biochem.*, **204**, 220 (1992).
4. Hjelmeland, J.M., and Chrambach, A., *Methods Enzymol.*, **104**, 305-318 (1984).
5. Doolittle, M.H., et al., *Analytical Biochemistry*, **195**, 364-368 (1991).

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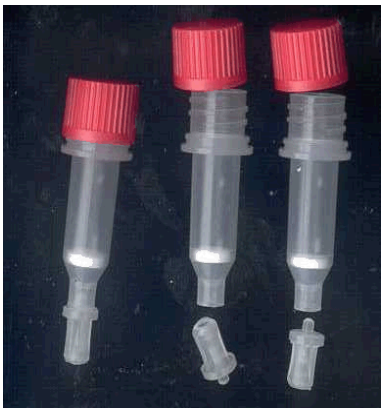
Troubleshooting Guide

Problem	Possible Cause	Solution
No signal is observed	Specific Antibody is not capable of immunoprecipitation.	Try a different antibody. Not every antibody, especially a monoclonal antibody, can immunoprecipitate. Add a bridging antibody.
	Antigen of interest not present in the sample.	Make sure the sample is appropriate. Prepare fresh lysates. Avoid using frozen lysates. Use a different lysing buffer. Use appropriate protease inhibitors in sample or increase their concentration.
	Washes are too stringent.	Reduce the number or washes. Avoid using high NaCl concentration. Use solutions that do not contain detergents.
	Incubation times are inadequate.	Try to prolong the incubation times.
	Interfering substance present in sample.	Lysates containing 2-mercaptoethanol, dithiothreitol (DTT), or other reducing agents may destroy antibody function and must be avoided. Excessive detergent concentrations can also interfere with the antibody-antigen interaction.
	Detection system is inadequate.	If Western blotting detection is used: Check primary and secondary antibodies. Check whether the transfer was adequate.
Background is too high	Proteins bind non specifically to Protein G.	Preclear the sample one or two additional times.
	Insufficient washes.	Increase the number of washes. Prolong the washes with 1× IP Buffer, incubating each wash for 15 minutes. Increase the salt and/or SDS concentrations in washing solutions. Centrifuge at low speed to avoid non-specific trapping of proteins during centrifugation of Protein G Agarose/antigen complexes.

Figure 1.

Spin Column



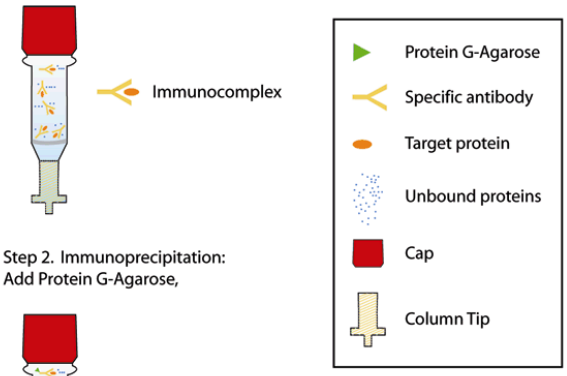
A. Column

B. Open column and snapped off tip

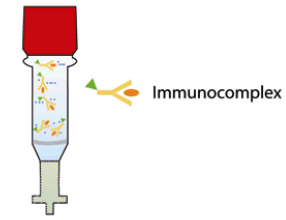
C. Inverted broken tip serves as column closure

Schematic of procedure

Step 1. Formation of antigen-antibody complex:
Mix cell lysate with antibody



Step 2. Immunoprecipitation:
Add Protein G-Agarose,



Step 3. Removal of non-specific binding: Snap off column tip, place in microfuge tube. Spin and wash extensively.

Step 4. Elution of the immunoprecipitated proteins
(Inverted broken tip serves as column closure)