Product Information

Anti-HA Immunoprecipitation Kit

IP0010

Storage Temperature: 2–8 °C

Product Description

The Anti-HA Immunoprecipitation Kit is specially designed to allow maximal recovery of HA-tagged proteins in the immunoprecipitates. The whole process is performed in mini-spin columns, instead of in microcentrifuge tubes, thus combining convenient washing of the antigen-antibody-bound beads with minimal variability between experiments.

The kit has several advantages:

- The use of Anti-HA-coupled beads ensures high specific binding to HA-tagged fusion protein.
- No preliminary steps and calibrations are needed when using the Anti-HA antibody directly coupled to beads.
- The use of spin columns ensures minimal loss of Anti-HA-bound beads during washing.
- Ready-to-use cell lysis reagent and 10X concentrated immunoprecipitation buffer.
- The CelLytic™ M Cell Lysis Reagent provides a high yield, and efficient and rapid lysis of mammalian cells.

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 can then be separated from the mixture by centrifugation. IP followed by SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE) and immunoblotting is routinely used in a variety of applications to measure the molecular masses of protein antigens, study protein-protein interactions, determine specific enzymatic activity, monitor protein post-translation 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 assays

- 10X IP Buffer: 40 mL (I5779)
- Anti-HA-Agarose: 0.5 mL (A2095)
- CelLytic™ M Cell Lysis Reagent: 50 mL (C2978)
- Spin Columns with Caps: 50 each (S3563)
- Collection Tubes (Polypropylene, 2 mL): 50 each (T7813)

Reagents Used (Not Provided)

- Microcentrifuge tubes
- Protease inhibitor cocktails:
  - P2714 – for general use, includes EDTA
  - P8340 – for use with mammalian cell and tissue extracts
  - P8465 – for use with bacterial cell extracts, includes EDTA
  - P8215 – for use with fungal and yeast extracts
- Sample Buffer, Laemmli 2X (S3401)
- Optional: DPBS (D8537) or TBS (T5030)

Precautions and Disclaimer

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

Preparation Instructions

1. 1X IP Buffer:

   For ten IP reactions, dilute 6.5 mL of 10X IP Buffer with 58.5 mL of ultrapure water to make 65 mL of 1X IP Buffer. For one IP reaction, dilute 0.7 mL of the 10X IP Buffer with 6.3 mL of ultrapure water to make 7 mL of 1X IP Buffer. The 0.1X IP Buffer is prepared by making a 10-fold dilution of the 1X IP Buffer with water. Each IP reaction requires 0.7 mL of 0.1X IP Buffer.
2. Prepare 1X Laemmli Sample Buffer by mixing the Sample Buffer, Laemmli 2X with an equal volume of water.

Storage/Stability

- Upon receipt store CelLytic™ M Cell Lysis Reagent (C2978), spin columns, and collection tubes at room temperature. Store the remaining reagents at 2–8 °C. **Do not freeze**.
- CelLytic™ M Cell Lysis Reagent (C2978) may appear cloudy after an extended period of storage. Product performance is unaffected and may be used, as is, without further filtration or clarification.

Procedure

Lysis of Mammalian Cells

The volume of CelLytic™ M Cell Lysis Reagent to be added to the cells varies according to plate size. In general, suggested working volumes are: 500-1000 µL for a 100 mm plate and 200-400 µL for a 35 mm plate.

A protease inhibitor cocktail may be added to the CelLytic™ M Cell Lysis Reagent.

Wash cells and treat with CelLytic™ M Cell Lysis Reagent.

- For adherent cells: Remove the growth medium from the cells to be assayed. Rinse the cells once with DPBS, being careful not to dislodge any of the cells. Discard the DPBS. Add appropriate volume of CelLytic™ M Cell Lysis Reagent.
- For cells in suspension: Collect cells in an appropriate centrifuge tube. Centrifuge for 5 minutes at 450 x *g*. Decant and discard the supernatant. Wash the cells once with DPBS and centrifuge for 5 minutes at 450 x *g*. Decant and discard the supernatant.
- Resuspend the cell pellet in the recommended volume of CelLytic™ M Cell Lysis Reagent.

3. Incubate the cells for 15 minutes on a shaker.
4. Collect the cell lysate.
   - For adherent cells: remove cells from plates (cell scraping may increase total protein yield).
   - For cells in suspension, skip to step 4.
5. Centrifuge the lysed cells for 15 minutes at 12,000–20,000 x *g* to pellet the cellular debris.
6. Remove the protein-containing supernatant to a chilled test tube.

   Note: For long-term storage it is recommended to store the lysate at –70 °C.

Immunoprecipitation of HA-Tagged Fusion Protein

See Figure 1 for a flow-chart of the immunoprecipitation procedure.

1. Thoroughly suspend the vial of Anti-HA-Agarose to make a uniform suspension of the resin. Add the indicated amounts (Table 1) of each component into an empty spin column.

Table 1. Components of immunoprecipitation reaction.

Component	Amount	Comments
Cell Lysate	100-600 µL	Amount depends on HA-tagged protein abundance (See Notes below)
Anti-HA-Agarose suspension	10 µL	(See Notes below)
1X IP Buffer	Complete volume to 600 µL	

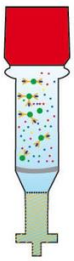
Note: It is convenient to set a negative control tube that contains cell lysate of non-transfected cells in order to identify non-specific binding of proteins to Anti-HA-Agarose.

Addition of 10 µL Anti-HA-Agarose per test is the recommended basic protocol. Optimal amounts for maximal detection and minimal signal-to-noise ratio should be determined experimentally.

2. Cap the spin column and incubate for 1 hour to overnight at 4 °C, mixing the sample head-over-tail. Note that longer incubations may lead to an increase in non-specific binding to the beads.

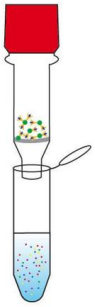
   Break off the tip of the spin column (Figure 2.B). Do not discard the tip. The inverted tip serves in later steps as a closure to the column (Sample Preparation for SDS-PAGE, step 2, Figure 2.C).
3. Insert each column into one of the 2 mL collection tubes supplied.
4. Spin in a microcentrifuge at 12,000 x *g* for 15–30 seconds at 4 °C. Discard the effluent.
5. Wash the beads in the spin column by resuspending in 700 µL of 1X IP Buffer.
6. Centrifuge at 12,000 x *g* for 15–30 seconds at 4 °C. Discard the effluent.
7. Repeat steps 6 and 7 five more times. The optimal number of washes may have to be determined experimentally.
8. Perform one last wash with 0.1X IP Buffer.

   Note: When exhaustive removal of detergent is required, 0.1X IP Buffer can be replaced by PBS or TBS in the last wash.
9. The sample is now ready for protein activity assays, which can be performed in the spin column, or for other downstream applications, such as analysis by SDS–PAGE.

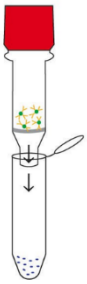


Immunocomplex

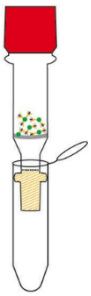
Step 1: Immunoprecipitation:
Lysate containing
HA-tagged protein +
Anti HA-Agarose beads.



Step 2: Removal of non specific binding: Snap off column tip, place in microfuge tube. Spin and wash extensively.



Add Elution Buffer
(Laemmli sample buffer),
open tip and spin



Clean Tube

Step 3: Elution of immunoprecipitate proteins (inverted broken tip serves as column closure).

Anti HA-Agarose beads

HA fusion protein

Unbound protein

Cap

Column

Figure 1. Flow Chart of Immunoprecipitation procedure.

Sample Preparation for SDS-PAGE

1. Centrifuge the spin column at 12,000 x *g* for 15–30 seconds at 4 °C.
2. Cap the bottom of the spin column tightly with the inverted column tip (Figure 2.C).
3. Add 20–100 µL of 1X Laemmli sample buffer to the column.
4. Mix the beads gently (no vortexing) in order to avoid spreading the beads on the column walls.
5. Cap the top of the column and insert into a new microcentrifuge tube. Transfer to a heating block preheated to 95 °C.
6. Heat sample for 5 minutes. Be sure that the Anti-HA-Agarose complex is within the heating chamber.
7. Open the column tip, insert the column back into the microcentrifuge tube, and centrifuge at 12,000 x *g* for 30 seconds.

   Note: Before opening the column tip, release vacuum by loosening the cap.

Figure 2. (**A**) Column, (**B**) Open column and snapped off tip and (**C**) Inverted broken tip serves as column closure.

References

1. Current protocols in Molecular Biology, Ausubel, F.M., et al., eds., John Wiley & Sons, Inc (1999).
2. Antibodies: A Laboratory Manual, Harlow, E., and Lane, D., eds., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1988).
3. Hjelmeland, J.M., and Chrambach, A., Methods Enzymol., 104, 305-318 (1984).
4. Doolittle, M.H., et al., Anal. Biochem., 195, 364-368 (1991).

Troubleshooting Guide

Problems	Possible cause	Solution
Little or no HA-tagged protein is observed.	Minimal or no HA-tagged protein expression in the sample.	Verify expression by Western blot analysis or immunocytochemistry.
	HA-tagged fusion protein is degraded.	Prepare fresh lysates. Avoid using frozen lysates. Use appropriate protease inhibitors in sample or increase their concentration.
	Washes are too stringent.	Reduce the number of washes. Wash with PBS or TBS.
	Interfering substance present in sample.	Lysates containing dithiothreitol, 2-mercapto-ethanol, or other reducing agents will destroy antibody function and must be avoided. Extremes in pH and excessive detergent concentrations can also interfere with the antibody-antigen interaction.
	Detection system is inappropriate.	For Western blot detection: • Check primary and secondary antibodies. • Verify protein transfer was efficient. • Check substrate used for detection.
Background is too high.	Insufficient washes	Increase the number of washes with 1X IP Buffer, and/or incubate each wash for 15 minutes.
	High protein concentration in the extract.	Calibrate the system with different amounts of agarose using a fixed amount of protein, and/or different amounts of protein using a fixed amount of agarose beads.
	Detection system is not calibrated.	For Western blot: • Check concentrations of primary and secondary antibodies. • Include control without addition of primary antibody.

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