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A Geno Technology, Inc. (USA) brand name

Classic Immunoprecipitation

Utilizes Protein A/G Agarose for Antibody Binding

(Cat. # 786-637)

INTRODUCTION 3
ITEM(S) SUPPLIED (CAT. # 786-637) 3
STORAGE CONDITIONS 3
ADDITIONAL ITEM(S) REQUIRED 3
IMPORTANT INFORMATION 4
PROTOCOL 4
LYSIS OF CELL SUSPENSIONS 4
LYSIS OF ADHERENT MAMMALIAN CELLS 5
PRECLEAR THE LYSATES 5
IMMUNE COMPLEX PREPARATION 5
IMMUNE COMPLEX CAPTURE 6
IMMUNE COMPLEX ELUTION FOR SDS-PAGE AND/OR WESTERN BLOT ANALYSIS 6
IMMUNE COMPLEX ELUTION FOR ENZYMATIC AND/OR FUNCTIONAL ASSAYS 6
TROUBLESHOOTING 7
RELATED PRODUCTS 7

INTRODUCTION

Immunoprecipitation (IP) is one of the most useful immunochemical techniques. IPs are routinely used to determine the presence and quantity of an antigen, molecular weight of a polypeptide, rate of synthesis or degradation, identify certain post translational modifications and interactions with other proteins, nucleic acids and ligands. IPs consist of four main steps:

1. Labeling of the antigen (Optional step)
2. Release of antigen by cell lysis
3. Formation of antibody-antigen complexes
4. Purification of the immune complexes.

G-Biosciences Classic Immunoprecipitation kit contains all the reagents necessary to complete all aspects of immunoprecipitation, with the exception of labeling. The kit is designed for effective immunoprecipitations using <10µg antibody. The kit is suitable for 50 reactions using 10µl Immobilized Protein A/G Agarose.

ITEM(S) SUPPLIED (Cat. # 786-637)

Description	Size
Collection Tube, 2ml	100
Control Agarose (4% agarose)	2ml
Elution Buffer	5ml
Immobilized Protein A/G Agarose	0.55ml resin
Mammalian Cell PE LB™	100ml
SDS-PAGE Sample Loading Buffer [2X]	2.5ml
Spin Column	50
Caps	50

STORAGE CONDITIONS

Shipped at ambient temperature. Upon arrival, store at 4^{o}C.

ADDITIONAL ITEM(S) REQUIRED

- 1X PBS
- Centrifuge tubes (1.5ml)

IMPORTANT INFORMATION

- Perform all steps at 4°C, unless stated otherwise.
- Perform centrifugations for 60 seconds at 1,000-2,000xg. Excessive speeds or times may result in resin clumping making it difficult to resuspend the resin.
- The immobilized antibody will elute with the antigen and will migrate at 50kDa (heavy chain) and 25kDa (light chain). If the presence of the antibody fragments interferes with antigen detection, we recommend using our Direct Immunoprecipitation kit (Cat. # 786-636).
- We recommend using an affinity purified antibody as serum, although compatible with this kit, will result in a lower antigen yield due to reduced specific antibody binding.
- For optimal results, we recommend including a protease inhibitor and phosphatase inhibitor cocktail in the lysis buffer.
  ProteaseArrest™ (Cat. # 786-108) and PhosphataseArrrest™ (Cat. # 786-450) are recommended.

PROTOCOL

Lysis of Cell Suspensions

1. Pellet the cells by centrifugation at 3,000x g for 5 minutes. Remove and discard the supernatant.
2. Wash the cell pellet once with 5-10ml PBS. Pellet the cells again by centrifugation. Remove and discard the PBS wash.
3. Add ice cold Mammalian Cell PE LB™ and suspend the cell pellet. Incubate on ice for 10-30 minutes with periodic mixing. Add 10µl Mammalian Cell PE LB™ for every 1mg wet cell pellet. For large wet cell pellets add ~10% the final volume of Mammalian Cell PE LB™, vortex to suspend the pellet and then add the remaining Mammalian Cell PE LB™.
   NOTE: *Freeze/ thaw cycles are not necessary for lysis; however, one or two freeze/ thaw cycles are not detrimental to the cell extract, and often ensure complete lysis.*
4. Centrifuge the suspension at 20,000x g for 30 minutes in a refrigerated centrifuge to pellet the cell debris. Collect the clear supernatant in a fresh tube and perform a protein concentration assay.

Lysis of Adherent Mammalian Cells

1. Remove the culture medium from the adherent cells.
2. Wash the cells once with PBS. Remove the PBS wash.
3. Add an appropriate volume of ice cold Mammalian Cell PE LB™ (see table) to cover the culture surface area.

Plate Size	Volume of Mammalian Cell PE LB™
96-well plate	50-100µl/well
24-well plate	100-200µl/well
6-well plate	200-400µl/well
60mm culture plate	250-500µl
100mm culture plate	500-1,000µl

4. Incubate on ice for 5-10 minutes with periodic mixing.
5. Transfer the lysate to a micro centrifuge tube. Centrifuge the suspension at 20,000x g for 30 minutes in a refrigerated centrifuge to pellet the cell debris. Collect the clear supernatant in a fresh tube and perform a protein concentration assay.

Preclear the Lysates

1. For every 1mg lysate, transfer 80µl homogenous Control Agarose Slurry to the lysate and seal the tube.
2. Incubate at 4°C for 30-60 minutes with mixing.
3. Centrifuge the tube for 60 seconds at 1,000-2,000xg and transfer the supernatant to a clean tube.

Immune Complex Preparation

NOTE: *The amount of lysate required and the length of incubation require optimization for each specific antibody-antigen system used. The following protocol uses the recommend 2-10µg affinity purified antibody.*

1. Add 2-10µg affinity purified antibody to 500-1000µg protein lysate.
2. Adjust the volume of the Immune Complex Solution to 300-600µl with Mammalian Cell PE LB™.
3. Incubate for 1 hour to overnight at 4°C with end-over-end mixing.

Immune Complex Capture

1. Swirl the bottle of Immobilized Protein A/G Agarose to obtain a homogenous slurry. Using a wide bore pipette, transfer 20µl slurry to a spin column.
2. Snap off the bottom end cap and retain for sealing the spin column. Centrifuge for 60 seconds at 1,000-2,000xg to remove the storage buffer.
3. Wash the resin with 100µl Mammalian Cell PE LB™ and discard the flow through. Repeat the wash step once.
4. Seal the bottom of the spin column and add the Immune Complex Solution to the spin column. Cap the top of the column and incubate at 4°C for 1 hour with end-over-end mixing.
5. Remove the bottom stopper and remove the cap from the spin column. Place the column in a 2ml collection tube and centrifuge for 60 seconds at 1,000-2,000xg. Collect the flow through and save until successful IP has been achieved.
6. Transfer the spin column to a clean tube and add 200µl Mammalian Cell PE LB™ and centrifuge for 60 seconds at 1,000-2,000xg. Repeat the wash three more times.

Immune Complex Elution for SDS-PAGE and/or Western Blot Analysis

1. Add 50µl SDS-PAGE Sample Loading Buffer [2X] to the spin column containing the immobilized immune complex.
2. Seal the top of the column, leave the bottom unsealed and place in a collection tube. Incubate at 100°C for 5-10 minutes.
3. Centrifuge for 60 seconds at 1,000-2,000xg to collect the eluate. Allow to cool to room temperature before loading of a polyacrylamide gel. Discard the spin column and resin.

Immune Complex Elution for Enzymatic and/or Functional Assays

1. Add 50µl Elution Buffer to the spin column containing the immobilized immune complex.
2. Incubate at room temperature for 10 minutes.
3. Centrifuge for 60 seconds at 1,000-2,000xg to collect the eluate. Perform additional elutions as needed and analyze eluates separately to ensure complete elution of antigen.
4. To neutralize the eluates, add 5µl 1M Tris, pH9.5 to the collection tubes.

TROUBLESHOOTING

Issue	Possible Cause	Suggested Resolution
No immunoprecipitation of antigen	Antigen levels to low to detect	Check protein expression and lysis efficiency by Western blotting
	Antibody not binding to the antigen	Try fresh aliquot of antibody, or a different antibody against the same antigen
	Components in Mammalian Cell PE LB™ may interfere with antigen-antibody interaction	Perform IP and washes in 1X TBS or 1X PBS
No functional activity of antigen in downstream applications	The low pH elution may have inactivated the antigen	Elute with a high salt and neutral pH elution buffer.

RELATED PRODUCTS

1. ***Direct Immunoprecipitation*** *(Cat. # 786-636): Utilizes our Amine Reactive HOOK™ Activated Agarose to covalently couple the antibody. The covalently coupled antibody is then retained on the agarose during elution preventing contamination of the immunoprecipitated antigen by the antibody.*
2. ***Cross-Link Immunoprecipitation*** *(Cat. # 786-639): Utilizes Protein A/G immobilized on agarose to bind antibody to immunoprecipitated proteins of interest. The supplied DSS protein cross-linker covalently couples the antibody to the resin removing downstream interference by the antibody.*
3. ***Co-Immunoprecipitation*** *(Cat. # 786-638): Utilizes our Amine Reactive HOOK™ Activated Agarose to covalently couple the antibody. The covalently coupled antibody is then retained on the agarose during elution preventing contamination of the immunoprecipitated antigen by the antibody. Used to study protein-to-protein interactions.*

For other related products, visit our website at www.GBiosciences.com or contact us.

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