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Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

Weitere Information auf den folgenden Seiten!
See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Protein A/G Coated Plate Immunoprecipitation Kit

Item No. 601970

www.caymanchem.com
Customer Service 800.364.9897
Technical Support 888.526.5351
1180 E. Ellsworth Rd · Ann Arbor, MI · USA

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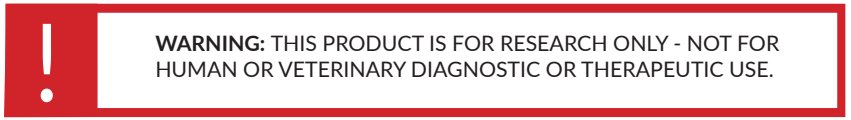
GENERAL INFORMATION

Materials Supplied

Item Number	Item Name	96 wells Quantity/Size	Storage Temperature
601975	Protein A/G-Coated 96-Well Strip Plate	2 plates	4°C
601977	Elution Plate	2 plates	RT
601973	IP Wash Buffer Detergent (10X)	1 vial/50 ml	4°C
601974	PBS (10X)	1 vial/50 ml	RT
601971	IP Elution Buffer	1 vial/50 ml	4°C
601972	IP Neutralization Buffer	1 vial/15 ml	4°C
400012	96-Well Cover Sheet	2 ea	RT

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.



Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

This kit may not perform as described if any reagent or procedure is replaced or modified.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Email: techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed in the Materials Supplied section (see page 3) and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. Adjustable pipettes; multichannel or repeating pipettor recommended
2. A source of pure water; glass-distilled water or deionized water is acceptable *NOTE: UltraPure Water is available for purchase from Cayman (Item No. 400000)*
3. SDS-PAGE and Western blotting materials if eluted samples are to be analyzed using these methods
4. Mass spectrometry (MS) instrumentation if eluted materials are to be analyzed using MS
5. Protein A and protein G resin (if pre-clearing is required)

INTRODUCTION

About This Assay

Cayman's Protein A/G Coated Plate Immunoprecipitation (IP) Kit provides a convenient method for the capture and concentration of target proteins from cell lysates, serum, and hybridoma culture media, as well as recombinant protein or recombinant antibody media, in a 96-well plate format. Following elution of the captured proteins from Protein A/G Coated 96-well strip plate, SDS-PAGE, Western blot, and mass spectrometry can be used for further analysis. This kit provides the following advantages over traditional tube- or resin-based IP methods:

1. The 96-well plate format allows for higher throughput by allowing the user to work with up to 96 samples
2. Much less time spent washing, as wells can be washed *via* multichannel pipette in seconds *versus* the minutes required for tube washes
3. No accidental transfer of resin beads between washes or during sample loading

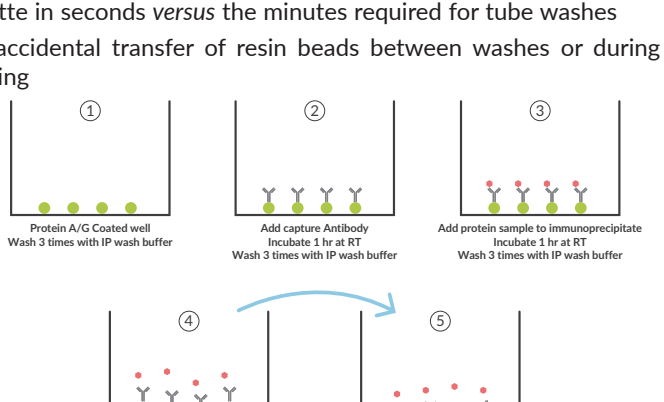


Figure 1. Schematics of the Protein A/G Coated Plate IP assay

Item Descriptions

1. **Protein A/G-Coated 96-Well Strip Plate**: Plates coated with a combination of protein A and protein G to allow for optimal antibody binding
   - Coating volume: 100 µl
   - Binding capacity: 2-5 µg/ml
2. **Elution Plate**: Non-binding plates intended for the receipt of immune complexes transferred from the Protein A/G-coated Strip Plates
3. **IP Wash Buffer Detergent (10X)**: Triton X-100-based detergent, which is an optimal washing and dilution buffer when diluted to 1X in PBS
4. **PBS (10X)**: PBS solution used to dilute IP Wash Buffer Detergent (10X)
5. **IP Elution Buffer**: Low pH buffer used to elute immune complexes from the Protein A/G-coated Plates
6. **IP Neutralization Buffer**: Neutralizes immune complexes eluted under low pH

PRE-ASSAY PREPARATION

1. IP Wash Buffer Preparation

Add the entire 50 ml of PBS (10X) (Item No. 601974) to 400 ml of pure water, followed by the addition of the entire 50 ml of IP Wash Buffer Detergent (10X) (Item No. 601973).

2. Antibody Preparation

It is recommended to use purified antibodies. Peptide/protein affinity-, protein A-, and protein G-purified materials yield the best results. Use of unpurified serum highly increases the possibility of non-specific background labeling. Dilute antibodies to 5-100 µg/ml (100 µl total volume per well) in IP Wash Buffer.

3. Sample Preparation

It is recommended to dilute the protein sample or lysate 1- to 50-fold in IP wash buffer (100 µl total volume per well).

Optional: Pre-clearing of sample to reduce non-specific background

- Apply 20 µl (resin bed volume) of a 1:1 ratio of protein A:protein G to each protein/lysate sample and mix by inversion for 1 hour at 4°C.
- Spin down at 500 x g, and remove lysate supernatant from beads.

ASSAY PROTOCOL

A. Binding of Antibody to the Plate

1. Remove the desired number of strips from the Protein A/G Coated 96-well strip plate and wash strips 3 times with 200 µl of IP Wash Buffer.
2. Add 100 µl of diluted antibody per well.
3. Cover the plate with the 96-well Cover Sheet (Item No. 400012) and incubate for 1 hour at room temperature or overnight at 4°C.

NOTE: If the antibody concentration is less than 5 µg/ml, it can be applied using multiple aliquots. For example, 300 µl of a 2 µg/ml antibody solution can be applied to the plate by: 1) Adding 100 µl of diluted antibody followed by a 20-60 minute incubation at room temperature, 2) Removing 100 µl from the well(s), discarding without washing, and adding an additional 100 µl of the diluted antibody for a 20-60 minute incubation at room temperature, and 3) Repeating step 2 for the final 100 µl of diluted antibody.

4. Wash strips 5 times with 200 µl of IP Wash Buffer.

B. Antigen Capture (Immunoprecipitation)

1. Add 100 µl of diluted protein sample or lysate to desired wells.

2. Cover wells with the 96-well Cover Sheet and incubate for 1 hour at room temperature or overnight at 4°C.

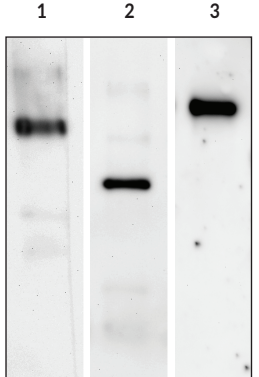
NOTE: Diluted protein samples (<0.1 µg/ml) can be applied using multiple aliquots. For example, 300 µl of a 50 ng/ml protein sample can be applied to the plate by: 1) Adding 100 µl of diluted sample followed by a 20-60 minute incubation at room temperature, 2) Removing 100 µl from the well(s), discarding without washing, and adding an additional 100 µl of the diluted sample for a 20-60 minute incubation at room temperature, and 3) Repeating step 2 for the final 100 µl of diluted sample.

3. Wash strips 5 times with 200 µl of IP Wash Buffer.

C. Immune Complex Elution

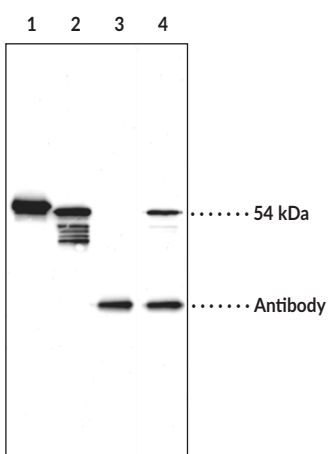
1. Add 22 µl of 6X SDS sample buffer to the number of wells needed in the Elution Plate.
2. Add 12 µl of IP Neutralization Buffer (Item No. 601972) to the wells with 6X SDS sample buffer.
3. Add 100 µl of the IP Elution Buffer (Item No. 601971) to the wells containing immune complex. Incubate for 15-30 seconds, and then immediately transfer eluted materials to wells of the Elution Plates containing 6x SDS Sample and IP Neutralization Buffer using a multichannel pipette.
4. Place Elution Plate strips in a 100°C oven for 5 minutes to reduce samples. Strips can then be stored at -20°C until analysis. Alternatively, samples can be transferred to tubes and reduced in a heat block.

Typical Results



Lane 1: Citrullinated Fibrinogen immunoprecipitated with Anti-Citrulline Monoclonal Antibody (Clone 1D9) (Item No. 30773) as the capture antibody and detected with the Fibrinogen (α chain) Polyclonal Antibody (Item No. 18033)
Lane 2: Vimentin immunoprecipitated with Vimentin Monoclonal Antibody (Clone 12E4) (Item No. 20197) as the capture antibody and detected with the Vimentin Polyclonal Antibody (Item No. 25341)
Lane 3: Human PCSK9 immunoprecipitated with PCSK9 (human) Polyclonal Antibody (Item No. 10007185) as the capture antibody and detected with PCSK9 (human) Monoclonal Antibody (Clone 15A6) (Item No. 10218)

Figure 2. Immunoprecipitation of proteins from buffer spiked samples. Capture antibodies were bound to the Protein A/G-Coated 96-Well Strip Plates, followed by elution and analysis using SDS-PAGE and Western blot.



Lane 1: Vimentin (human, recombinant) (Item No. 11234)
Lane 2: HEK293 lysate (control)
Lane 3: Eluted capture antibody control, Vimentin Polyclonal Antibody (Item No. 25341), without HEK293 lysate
Lane 4: Eluted Vimentin immunoprecipitation complex from HEK293 lysate with Vimentin Polyclonal Antibody (Item No. 25341) as the capture antibody

Figure 3. Immunoprecipitation of Vimentin from HEK293 lysate and Western blot analysis. SDS-PAGE, transferred to nitrocellulose, and detected by Western blotting with Vimentin Monoclonal Antibody (Clone 12E4) (Item No. 20197) and Goat Anti-Mouse IgG HRP conjugated secondary antibody (Item No. 10004302).

RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Target protein not detected	Low abundance in lysate	1. Increase the amount of antibody on the plate 2. Increase the amount of lysate applied to plate
Non-specific background too high	1. Non-specific binding of proteins to protein A/G 2. Antibody is not pure enough	1. Pre-clear sample using protein A/G prior to applying proteins to the protein A/G plate 2. Choose a different antibody with a higher purity or further purify antibody

NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.