INSTRUCTIONS

Thermo SCIENTIFIC

Pierce® Protein A/G Plate IP Kit

45350 | 1440.3

Number	Description
45350	**Pierce Protein A/G Plate IP Kit,** contains sufficient reagents to process 48 samples with the appropriate controls or 192 total immunoprecipitation reactions

Kit Contents:

Protein A/G Coated 96-well Strip Plate, 2 each

Binding Capacity: 2-3 µg/well

Coating Volume: 100 µl

Uncoated 96-well Strip Plate (white), 2 each

BupH™ Phosphate Buffered Saline Pack, 1 pack, results in 0.1 M phosphate, 0.15 M NaCl, pH 7.2 when reconstituted with 500 ml of ultrapure water

Surfact-Amps® X-100, 6 × 10 ml vials of 10% Triton® X-100

Elution Buffer 50 ml, pH 2.8, contains primary amines

Neutralization Buffer 12 ml, pH 8.5, contains primary amines

Sealing Tape for 96-well plates, 18 sheets

Storage: Upon receipt store at 4°C. Product shipped at ambient temperature.

Table of Contents

Introduction 1
Important Product Information 2
Additional Materials Required 2
Procedure for Plate Immunoprecipitation 2
A. Bind Antibody to the Protein A/G Plate 2
B. Capture Antigen (Immunoprecipitation) 3
C. Elute the Immune Complex and Prepare Sample for SDS-PAGE 3
Troubleshooting 4
Additional Information 4
Related Products 5
General References 5

Introduction

The Thermo Scientific Pierce Protein A/G Plate IP Kit contains all the necessary reagents to perform IPs in a 96-well microplate format. The Protein G or A/G coated plate can be used to either add the antibody directly to the plate followed by the antigen-containing material, or the antibody-antigen immune complexes may be prepared first and then added to the plate. After wells are washed to remove nonbound materials the immunoprecipitated protein is recovered using an elution buffer and is then analyzed by SDS-PAGE.

The Pierce Protein A/G Plate IP Kit enables many samples to be processed at the same time. Furthermore, this method produces consistent results with replicates and with repeat experiments. Tedious wash steps are eliminated, allowing a higher throughput mode of sample processing. The kit also can be used for co-immunoprecipitation (co-IP) experiments. The strip format of the coated plate is particularly useful when only part of the plate is needed. The combined use of the plate format with a multichannel pipettor allows better sample handling with reduced effort.

Important Product Information

- Because Protein A/G binds antibodies reversibly, the antibody will co-elute with the antigen and result in detection of the antibody and antigen on a gel or Western blot. If the antibody co-migrates with the antigen, IP samples may be electrophoresed using reducing conditions to change the antibody and antigen band patterns. By including the appropriate controls and evaluating samples using reducing and non-reducing conditions, the band(s) of interest can be identified by the process of elimination. For a Western blot, a primary antibody from a different species or a biotinylated antibody and enzyme-labeled streptavidin can be used to eliminate detection of the IP antibody.
- Perform all steps at room temperature unless otherwise indicated.
- Use laboratory tape to secure the strips on the frame to prevent strips from detaching during wash steps.
- For optimal results, use an affinity-purified antibody. Note that when using serum, the antibody that is specific for the target antigen may comprise only 1-2% of the total IgG in the serum and will result in low antigen yields. Typically, rabbit serum contains ~10-12 mg/ml IgG and mouse serum contains ~6-8 mg/ml IgG. Some species may contain up to 30 mg/ml IgG. The average total IgG concentration for ascites fluid is ~1-10 mg/ml. If using tissue culture supernatant (serum-free medium), the average IgG concentration is 0.05 mg/ml and may be too dilute to effectively capture enough antigen for analysis. For best results concentrate tissue culture supernatant to at least 0.5 mg/ml before forming the immune complex.
- The amount of antigen and incubation time needed are dependent on the antibody-antigen system used and will have to be optimized for each specific system.
- The antigen and antibody samples MUST NOT contain SDS.

Additional Materials Required

- Lane Marker Reducing Sample Buffer (5X) (Product No. 39000) or Lane Marker Non-Reducing Sample Buffer (5X) (Product No. 39001)

  Note: Other sample buffers may be used. Example sample buffers recipes are provided in the Additional Information Section.

Procedure for Plate Immunoprecipitation

Procedural Note: For best results use an antibody solution volume of 50-200 µl and an antigen sample volume equal to or greater than the antibody volume. Use an Elution Buffer volume that is equal to the antibody volume; however, because the Protein A/G coated volume is 100 µl, it is not advantageous to exceed this volume for elution.

A. Bind Antibody to the Protein A/G Plate

1. Prepare the IP Buffer by dissolving a BupH PBS Pack in 450 ml of ultrapure water. Add 5 vials (50 ml) of the Surfact-Amps X-100 to the PBS buffer. Mix well. For long-term storage add 0.05% sodium azide. Store IP Buffer at 4°C.
2. Determine the number of strips required and leave these strips in the plate frame. (For a suggested experimental design, see the Additional Information section). Secure strips in the frame with laboratory labeling tape to prevent strips from detaching during the procedure. Carefully remove the remaining strips and store them with desiccant in the original pouch. Seal the pouch and store at 4°C.

   Note: After completing the IP procedure, retain plate frame for the remaining strips. The strips cannot be reused.
3. Wash each well three times with 200 µl IP Buffer.
4. Dilute the antibody in IP buffer to 10-100 µg/ml and add the antibody solution to the wells (see Procedural Note at the beginning of this section for appropriate volumes to use).

   Note: If the antibody solution is dilute (i.e., < 1 µg/ml), add the antibody solution in three separate aliquots and incubate for 20 minutes each. For example, when using 500 µl of a dilute antibody solution, add 166 µl of the solution to the well and incubate for 20 minutes. Remove the incubated sample and repeat this procedure twice using the remaining solution.
5. Cover the strip wells with Plate Sealing Tape and incubate at room temperature for 1 hour.
6. Wash wells five times with 200 µl IP Buffer.

B. Capture Antigen (Immunoprecipitation)

1. Dilute the antigen sample or lysate 1:1 to 1:100 in IP buffer and add to appropriate wells (see Procedural Note for appropriate volumes to use).

   Note: If the antigen solution is dilute (i.e., < 0.1 µg/ml) and is in a large volume, add the antigen sample in three separate aliquots and incubate for 20 minutes each.

   Note: For co-IP applications, pre-mix the antigen and the corresponding protein to allow the protein:protein interaction to occur and then add it to the plate.

2. Cover wells with Plate Sealing Tape and incubate at room temperature for 1 hour.
3. Wash wells five times with 200 µl IP Buffer. Incubate the last wash for 5 minutes.

C. Elute the Immune Complex and Prepare Sample for SDS-PAGE

Caution: If native conditions will be used for gel electrophoresis, DO NOT transfer samples to the supplied polystyrene plate. Transfer the samples to labeled polypropylene tubes or polypropylene plates. Native sample buffer promotes protein binding to polystyrene plates and may result in sample loss.

1. Add sample buffer (see Additional Materials Required Section) to the wells of the white, uncoated collection strips using the same number of strips as used for the IP procedure. The final concentration of sample buffer should be 1X when the other components are added (see Table 1 for examples of volumes to add).

Table 1. Examples of sample preparations.

		Sample Buffer (µl)	
Elution Buffer (µl)	Neutralization Buffer (µl)	2X	5X
50	5	50	10
100	10	100	20

Note: Save the unused white collection strips in the resealable bag for future use.

2. Add Neutralization Buffer to the wells containing the sample buffer. Add a volume that is equal to 10% of the Elution Buffer volume to be used (Table 1).
3. Add Elution Buffer to the wells containing the immune complex. Incubate the wells for 10-15 seconds. The elution time MUST NOT exceed 1 minute.
4. Use a multichannel pipettor to transfer the eluted proteins to the white collection strips prepared in steps 1-2.
5. Store plate at 4°C until evaluation by SDS-PAGE using either of the following options:
   - Non-Reducing: Denature samples by heating the covered plate for 5 minutes in an oven pre-heated to 85-100°C. Allow plate to cool to room temperature before applying sample to the electrophoresis gel.
   - Reducing: If a reducing sample buffer was not used, reduce samples by adding 20 mM DTT or 10 mM TCEP or β-mercaptoethanol to the wells and mix. Place the covered plate in a 95°C oven for 5 minutes. Allow plate to cool to room temperature before applying sample to the electrophoresis gel.

Troubleshooting

Problem	Possible Cause	Solution
No antigen detected	Sample does not contain sufficient antigen to detect	Concentrate the sample before IP, or incubate the wells three times with the dilute antigen sample
	Antibody concentration is insufficient to capture the antigen	Concentrate the antibody or incubate the plate wells three times with the dilute antibody solution
	Incomplete cell lysis or the protein may not have been expressed	Verify protein expression and efficiency of lysis by SDS-PAGE analysis of the crude lysate
	Antibody cannot bind antigen	Obtain new antibody
	Elution buffer not efficient at disrupting antibody-antigen interaction	Wash plate with 1 M sodium borate buffer, pH 8.0 and use 3.5 M $MgCl_2$ to elute antigen (sample needs to be dialyzed before SDS-PAGE analysis)
	Antibody not bound by Protein G	Verify that the antibody binds Protein A/G or use Streptavidin Kit (Product No. 45360) with biotinylated antibody for the capture of the antigen
	Detection method not sensitive enough to detect antigen	Use a more sensitive detection method
		Use 5X Sample Buffer instead of 2X Sample Buffer so that the sample applied to the gel is more concentrated
	Antigen may not be binding to the membranes chosen for transfer	Use a different membrane for the protein transfer
Too many bands detected	Some bands may be the antibody	Perform recommended controls and/or increase number of washes
	Nonspecific binding of proteins to the plate	Add a blocking protein, such as BSA, to the antigen sample
		Centrifuge lysate at 10,000 × *g* for 30 minutes before adding to the plate

Additional Information

A. Suggested Experimental Design (samples and controls)

Control	Antibody	Antigen
Test	+	+
No Antigen	+	-
No Antibody	-	+
Protein G or A/G	-	-

B. SDS-PAGE Sample Buffer Recipes

Sample Buffer	Component	Volume
2X Native Tris-Glycine Sample Buffer	0.5 M Tris•HCl, pH 8.8	4.0 ml
	Glycerol	2.0 ml
	0.1% Bromophenol blue	0.5 ml
	Ultrapure water	3.5 ml
2X Tris-Glycine SDS Sample Buffer (denaturing, non-reducing)	0.5 M Tris•HCl, pH 6.8	2.5 ml
	Glycerol	2.0 ml
	10% (w/v) SDS	4.0 ml
	0.1% Bromophenol blue	0.5 ml
	Ultrapure water	1.0 ml

C. Please visit the our web site for additional information on this product including the following item:

- Tech Tip: Binding characteristics of Proteins L, A, G and A/G to immunoglobulins

Related Thermo Scientific Products

88013	**Nitrocellulose Membrane, 0.2 µm,** 7.9 × 10.5 cm, 15 sheets/pkg
88114	**PDVF Membrane, 0.45 µm,** 7.9 × 10.5 cm, 15 sheets/pkg
34075	**SuperSignal® West Dura Extended Duration Substrate,** 100 ml
34080	**SuperSignal West Pico Chemiluminescent Substrate,** 500 ml
24612	**Silver Stain Kit II**
24590	**GelCode™ Blue Stain Reagent,** 500 ml
15033	**Microplate Strip Well Ejector,** 1/pkg.
20490	**TCEP•HCl,** 1 g, potent, water-soluble, odorless reducing agent
77720	**Bond-Breaker TCEP Solution, Neutral pH,** 5 ml

General References

Bennet, R.M., *et al.* (1988). The production and characterization of murine monoclonal antibodies to a DNA receptor on human leukocytes. *J. Immunol.* **140:**2937-42.

Harlow, E. and Lane, D. (1988). Antibodies: A Laboratory Manual. Cold Spring Harbor Laboratory: Cold Spring Harbor, New York.

Kerr, M.A. and Thorpe, R. (1994). Immunochemistry LabFax. BIOS Scientific Publishers, Ltd: Oxford, UK.

Reeves, H.C., Heeren, R. and Malloy, P. (1981). Enzyme purification using antibody crosslinked to protein A agarose: application to *Escherichia coli* NADP-isocitrate dehydrogenase. *Anal. Biochem.* **115:**194-6.

Schneider, C., *et al.* (1982). A one-step purification of membrane proteins using a high efficiency immunomatrix. *J. Biol. Chem.* **257:**10766-9.

Sisson, T.H. and Castor, C.W. (1990). An improved method for immobilizing IgG antibodies on protein A-agarose. *J. Immunol. Meth.* **127:**215-20.

Taylor-Papadimitriou, J., Shearer, M. and Griffin, D. (1987). Epitopes of human interferon-γ defined by the reaction of monoclonal antibodies with an interferons and interferon analogues. *J. Immunol.* **139:**3375-91.

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