INSTRUCTIONS

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Pierce® Streptavidin Plate IP Kit

45360

1439.3

Number	Description
45360	**Pierce Streptavidin Plate IP Kit,** contains sufficient reagents to process 48 samples with the appropriate controls or to perform 192 total immunoprecipitation reactions

Kit Contents:

High-Binding Capacity Streptavidin Coated 96-well Strip Plate, 2 each

Binding Capacity: 3-5 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 sodium chloride, pH 7.2 when reconstituted with 500 ml of ultrapure water

Biotin Blocking Buffer, 2 × 15 ml, contains 2 mM biotin

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.

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Introduction

The Thermo Scientific Pierce Streptavidin Plate IP Kit contains all the necessary reagents to perform IPs in a 96-well microplate format. In this method, the biotinylated antibody is directly added to the plate followed by the antigen-containing material. After wells are washed to remove nonbound materials, the immunoprecipitated protein is recovered using an elution buffer and then analyzed by SDS-PAGE.

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

Important Product Information

- Antibody bands may be detected during Western blot analysis if too much biotinylated antibody is added to the wells and a biotinylated antibody and Streptavidin-HRP conjugate or Streptavidin-AP conjugate is used for the Western blot. For best results, add < 150 ng/50 µl (i.e., 30 ng/µl) of the biotinylated antibody to the wells. Furthermore, using a non-biotinylated primary antibody for the Western blot will reduce detection of nonspecific antibodies.
- 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 biotinylated antibody. Note that when using biotinylated 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. On average, rabbit serum contains ~10-12 mg/ml IgG and mouse 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.
- The amount of biotinylated antibody, antigen and incubation time needed is 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.
- Negative controls are absolutely necessary for generating significant results. Controls will help identify and eliminate false positives caused by nonspecific binding of proteins to the streptavidin plate. Please refer to the Additional Information section for suggested controls.
- For a list of suggested reagents to biotinylate antibodies, please refer to the Additional Information section.

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 a biotinylated 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 streptavidin coated volume is only 100 µl, it is not advantageous to exceed this volume for elution.

A. Antibody Binding to the Streptavidin Coated 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 secure strips in the frame with laboratory labeling tape. (For a suggested experimental design, see the Additional Information section.) 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. Incubate the last two washes for 5 minutes each.
4. Dilute the biotinylated antibody in IP buffer to 5-30 ng/µl 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., < 0.1 µg/ml), add the antibody solution in three separate aliquots and incubate for 10 minutes each. For example, when using 500 µl of dilute antibody solution, add 166 µl of the solution to the well and incubate for 10 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 30 minutes.
6. Wash wells three times with 200 µl IP Buffer.

B. Biotin Blocking

1. Add 100 µl of the Biotin Blocking Buffer to all wells and incubate for 60-90 seconds.
2. Wash the wells three times with 200 µl IP Buffer.

C. Antigen Capture (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. Remove Sealing Tape and wash wells five times with 200 µl IP Buffer. Incubate the last two washes for 5 minutes each.

D. Immune Complex Elution and Sample Preparation 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 the 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 that is 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 2-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	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 lysis efficiency 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 the streptavidin plate	Verify that the antibody was biotinylated and that excess biotinylation reagent was removed
	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	The IP antibody is being detected	Optimize the amount of biotinylated antibody added to the wells
		Perform recommended controls and/or increase number of washes
		Use a non-biotinylated primary antibody for the Western blot detection
	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. Recommended Experimental Design (samples and controls)

Some suggested controls are as follows:

Control	Biotinylated Antibody	Antigen
Test	+	+
No Antigen	+	-
No Antibody	-	+
Streptavidin	-	-

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. To biotinylate antibodies in the laboratory, use one of the following reagents:

- Sulfo-NHS-LC-Biotin (Product No. 21335): This reagent biotinylates through primary amine groups and is most commonly used to biotinylate antibodies. This reagent is also available in a kit format (Product No. 21420).
- Biotin-BMCC (Product No. 21900): This reagent biotinylates the IgG through the sulfhydryl groups that are produced by reduction of the antibody with 2-mercaptoethylamine•HCl (Product No. 20408) or introduced through the primary amine groups of the antibody by *N*-succinimidyl-S-acetylthioacetate (SATA, Product No. 26102) or 2-iminothiolane•HCl (Traut's Reagent, Product No. 26101)
- Biotin-LC-Hydrazide (Product No. 21340): This reagent biotinylates the IgG through the carbohydrate moieties on the Fc region.

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

24597 **Color Silver Stain Kit**

24612 **Silver Stain Kit**

24590 **GelCode® Blue Stain Reagent,** 500 ml

20490 **TCEP•HCl,** 1 g, potent, water-soluble, odorless reducing agent

77720 **Bond-Breaker™ TCEP Solution, Neutral pH,** 5 ml

15033 **Microplate Strip Well Ejector,** 1/pkg.

General References

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Current versions of product instructions are available at *www.thermo.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.

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