

Pierce[®] HA Tag IP/Co-IP Kit

26180

2288.0

Number	Description
26180	Pierce HA Tag IP/Co-IP Kit, contains sufficient components for 25 immunoprecipitations or co-immunoprecipitations involving HA-tagged proteins Kit Contents: HA-tagged Positive Control (26180X), 500µL, 1mg/mL <i>E. coli</i> extract containing HA-tagged GST-PI3K-SH2 domain Application Set (26180Y): Anti-HA Agarose, 250µL settled beaded agarose coupled with 875µg of antibody and supplied as 50% slurry (<i>i.e.</i>, 500µL total volume) in PBS and 0.05% sodium azide as a preservative BupH™ Tris Buffered Saline Pack, 1 pack, contains 25mM Tris, 0.15M NaCl; pH 7.2 when reconstituted with 500mL of ultrapure water Elution Buffer, 50mL, pH 2.8 Lane Marker Non-Reducing Sample Buffer (5X), 5mL, contains 0.3M Tris•HCl, pH 6.8, 5% SDS, 50% glycerol, Lane Marker Tracking Dye Pierce Spin Columns Accessory Pack, 27 columns with pre-inserted frit and top and bottom caps Collection Tubes and Caps Accessory Pack, 100 graduated 2mL tubes and plug caps Storage: Upon receipt, store the HA-tagged Positive Control at -80°C. Alternatively, store the Positive Control for up to six months at -20°C. The HA-tagged Positive Control is shipped with dry ice. Store the Application Set at 4°C. The Application Set is shipped at ambient temperature.

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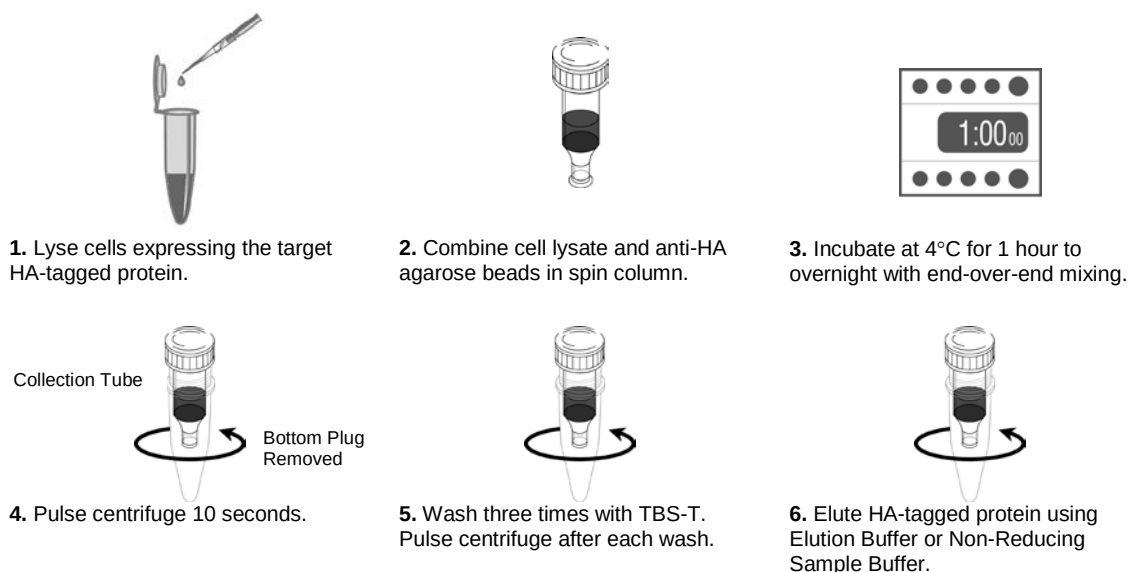
Introduction

The Thermo Scientific Pierce HA-Tag IP/Co-IP Kit provides a simple and fast method to study HA-tagged proteins, with advantages over the traditional IP procedure using protein A/G agarose. The high affinity anti-HA antibody-coupled agarose enables immunoprecipitation (IP) of HA-tagged proteins or co-immunoprecipitation (co-IP) of their interacting partners without antibody contamination. The microcentrifuge spin columns provided with the kit handle small volumes of anti-HA agarose and allow the entire procedure to be performed in a single spin column, preventing anti-HA agarose loss between washes. The spin columns further enhance recovery by efficiently separating buffer from the anti-HA agarose.¹

Epitope tagging is a widely used method that enables surveillance of the resultant fusion protein with tag-specific rather than protein-specific antibodies.^{2,3} Immunoprecipitation of epitope-tagged proteins can be performed to determine the protein's cellular localization, to study post-translational modifications or to detect interactions between tagged proteins and other proteins. A common epitope tag is the HA tag, which consists of nine amino acids (YPYDVPDYA) derived from the human influenza virus hemagglutinin (HA) protein. Immunoprecipitation of HA-tagged proteins using anti-HA antibodies distinguishes the expressed protein from the natively expressed protein population.

The Pierce HA-Tag IP/Co-IP Kit uses a high-affinity anti-HA antibody that enables the isolation and identification of tagged protein regardless of its expression level. The covalent linkage between the antibody and the agarose results in a final immunoprecipitated product free of antibody contamination. The HA-tagged positive control can be used to verify IP and anti-HA agarose performance (see Appendix for example data).

Procedure Summary



Important Product Information

- For best results, determine optimal conditions for HA-tagged fusion protein expression before attempting IP.
- Include a non-transfected lysate as a negative control to identify nonspecific binding of proteins to the anti-HA agarose. The HA-tagged Positive Control assists in verifying whether the anti-HA agarose can successfully capture the HA-tagged protein.
- The buffer provided in the kit allows complete flexibility to determine optimal conditions for isolating interacting proteins. Characteristics of interacting proteins may require alteration of the binding and wash buffers. Protein cofactors, divalent cations, detergents or additional salts may be added.
- For optimal results in obtaining HA-tagged proteins, add protease inhibitors (e.g., Thermo Scientific Halt Protease Inhibitor Single-Use Cocktail, EDTA-Free, Product No. 78425) when preparing any lysate.

Additional Materials Required

- Microcentrifuge capable of $16,000 \times g$
- Protease inhibitors (e.g., Product No. 78425 or 78430)
- Tween[®]-20 Detergent (Product No. 28320)
- End-over-end rocker or rotator
- Heat block
- 0.2 μ m, 500mL filter apparatus
- 1M Tris, pH 9.5
- 1M DTT (Product No. 20291) or β -mercaptoethanol (Product No. 35602)

Material Preparation

- **Tris-buffered saline (TBS):** Reconstitute contents of the TBS pack with 500mL of ultrapure water. Filter sterilize solution using a 0.2 μ m filter apparatus and store at 4°C. Final concentration is 25mM Tris•HCl, 0.15M NaCl; pH 7.2.
- **Non-Reducing Sample Buffer (2X):** Equilibrate the Non-Reducing Sample Buffer (5X) to room temperature. Gently mix the Sample Buffer by inverting the bottle 5-10 times. The Sample Buffer is viscous and may require that the pipette tip be “snipped” to allow the solution to be drawn into the tip. Dilute Sample Buffer by adding 600 μ L ultrapure water to 400 μ L Sample Buffer. Pipette up and down to mix. Store this solution at 4°C for up to 1 year.

Procedure for IP/Co-IP of HA-tagged Protein

A. IP/Co-IP

Note: The amount of lysate needed and incubation time are dependent upon the expression level of the HA-tagged protein and requires optimization for each specific system. For co-IP experiments, the buffer system must be optimized to maintain the protein:protein interaction.

1. Place the bottom plugs on the spin columns and ensure that the inserted porous disc in each column is seated properly at the bottom. Add the appropriate amount of lysate to the spin columns.
Note: For initial optimization, use 200 μ L lysate (400-600 μ g total protein) from a 60mm plate of confluent cells (see Figure 1). Maximum volume for the spin column is 850 μ L.
2. To set up a positive control, use 50 μ L of the HA-tagged Positive Control diluted in 150 μ L TBS.
3. Thoroughly resuspend the anti-HA agarose by inverting the vial several times immediately before dispensing. Do not vortex. Dispense 20 μ L of anti-HA agarose slurry (= 35 μ g of antibody) into each labeled spin column using a wide-bore pipette tip. Screw on the cap.
4. Incubate with gentle end-over-end mixing for at least 1 hour at 4°C; typical incubation times range from 2 hours to overnight.
5. Prepare a wash solution of TBS plus 0.05% Tween-20 (TBS-T) Detergent. For each spin column, prepare approximately 3mL of wash solution.
6. Loosen the top cap on the column, and then remove the bottom plug. Place a collection tube under the column and pulse centrifuge for 10 seconds. Discard the flow-through or save for future analysis.
7. Add 0.5mL of TBS-T to each column. Loosely screw on the cap and gently invert the column with the collection tube 2-3 times. Pulse centrifuge for 10 seconds. Discard the wash or save for future analysis.
8. Repeat step 7 two additional times.

B. Elution of HA-tagged Protein

Note: If the eluted HA-tagged protein will be used for functional applications, use Elution Protocol 1 to elute the protein. If the protein is sensitive to the low pH, use the Gentle Elution Buffer (Product No. 21027). For electrophoretic analysis, use Elution Protocol 2.

• Elution Protocol 1:

1. Place the spin column in a new collection tube. Add 10 μ L of Elution Buffer to the anti-HA agarose, loosely screw on the cap and gently tap the tube to mix. Pulse centrifuge for 10 seconds.
Note: It is not necessary to place the bottom plug on the spin column for this step.
2. Repeat step 1 two additional times. The three elutions may be recovered and pooled in one collection tube.
Note: Neutralize the eluent immediately by adding 1 μ L of 1M Tris, pH 9.5 per 20 μ L of Elution Buffer.
3. For reducing gel analysis, prepare reducing sample buffer by adding 10 μ L of 1M DTT or 5 μ L of β -ME to the 40 μ L of Lane Marker Non-Reducing Sample Buffer (5X).
4. Add 7.5 μ L of the prepared reducing sample buffer to 30 μ L elution sample. Heat sample at 95-100°C for 5 minutes.

• Elution Protocol 2:

1. Place the spin column in a new collection tube. Add 25 μ L of 2X Non-Reducing Sample Buffer (see Material Preparation Section) to the anti-HA agarose, loosely screw on the cap and gently tap the tube to mix.
Note: It is not necessary to place the bottom plug on the spin column for this step.
2. Heat the spin column/tube assembly at 95-100°C on a heat block for 5 minutes. Pulse centrifuge for 10 seconds.
Note: Using Non-Reducing Sample Buffer can minimize interference from co-eluting antibody fragments.
3. To prepare the sample for reducing SDS-PAGE, add 2-3 μ L of 1M DTT or 1-2 μ L of β -ME to the 25 μ L sample.
Note: The eluted HA-tagged Positive Control can be detected by coomassie or silver staining (see Related Thermo Scientific Products). For more sensitive detection methods such as Western blotting, dilute the eluted HA-tagged positive control 10- to 50-fold (0.2-0.5 μ L elution is usually sufficient for analysis).

Troubleshooting

Problem	Cause	Solution
Little or no HA-tagged protein is detected	Tagged protein degraded	Include protease inhibitors (e.g., Product No. 78425 or 78430) in the lysis buffer
		Use new lysate or lysate stored at -80°C
	No or minimal tagged protein was expressed	Verify protein expression by SDS-PAGE or Western blot analysis of the lysate using HA-tagged Positive Control as a reference
		Increase the amount of lysate used for IP/Co-IP
Failure to Co-IP interacting protein	Wash conditions were too stringent for the weak or transient interaction	Reduce the number of washes and/or lower the ionic strength of wash buffer
	Interacting protein was expressed at a low level	Apply additional protein sample or use a more sensitive detection system
	The buffer system was not optimal for the protein:protein interaction	Optimize the Co-IP buffer

Appendix

A. Immunoprecipitation of HA-tagged SRF (serum response factor)

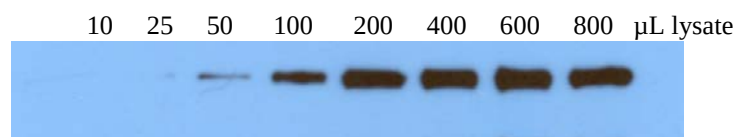


Figure 1. Immunoprecipitation of HA-tagged SRF (serum response factor) from a cell lysate. Human embryonic kidney cells (293) were transfected with pCMV-HA-SRF for 40 hours. Cells were lysed in Thermo Scientific M-PER Protein Extraction Reagent (200μL/60mm plate) containing protease inhibitors. Various amounts of lysate were incubated with 6μL anti-HA agarose slurry at 4°C overnight. HA-SRF was eluted with 25μL non-reducing sample buffer, and 10μL of the eluted HA-SRF was separated by SDS-PAGE, transferred to a nitrocellulose membrane and probed with anti-HA antibody. Pierce Goat Anti-Mouse IgG (H+L), Peroxidase Conjugated (Product No. 31430; 1:100,000 dilution) was used as the secondary antibody. The membrane was incubated with SuperSignal® West Pico Chemiluminescent Substrate (Product No. 34080) and exposed to X-ray film.

B. Co-immunoprecipitation of active Rac1 with HA-tagged Pak1-PBD

Small GTPase Rac1 cycles between active GTP-bound form and inactive GDP-bound form. Upon activation, Rac1 activates p21-activated protein kinase 1 (Pak1) through binding to the p21-binding domain (PBD) of Pak1. When Rac1 mutant (Q61L) is co-expressed with HA-tagged Pak1-PBD, it is co-immunoprecipitated with HA-tagged Pak1-PBD using anti-HA agarose.

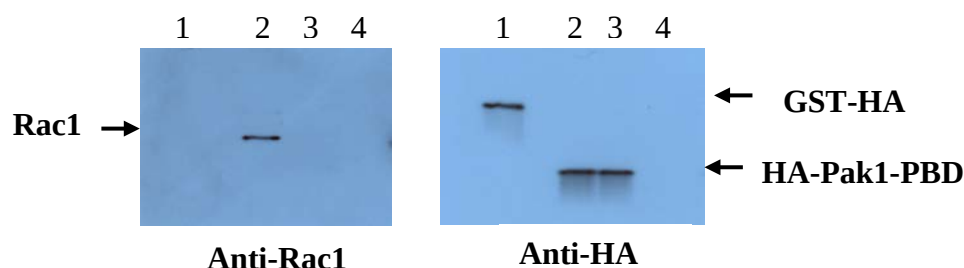
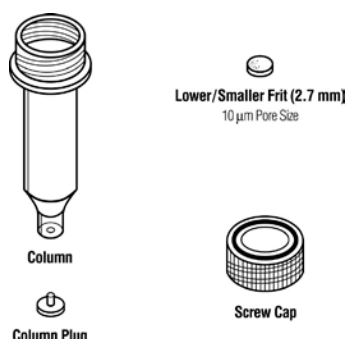


Figure 2. Co-IP of active Rac1 with HA-tagged Pak1-PBD (p21 binding domain). Human 293 cells were transfected with HA-Pak1-PBD alone or co-transfected with constitutively activated Rac1 (Q61L) in 293 cells. Cells were lysed in the buffer containing 25mM Tris, pH 7.2, 150mM NaCl, 5mM MgCl₂, 0.5% NP-40, 1mM DTT, 5% glycerol and protease inhibitors (500μL/100mm plate). Anti-HA agarose slurry (6μL) was incubated with 50μL positive control lysate from the kit (Lane 1) or 500μL cell lysate from Rac1 (Q61L) and HA-Pak1-PBD co-transfected cells (Lane 2), HA-Pak1-PBD transfected cells (Lane 3) or non-transfected cells (Lane 4). IP and co-IP reactions were performed at 4°C overnight. IP and Co-IP products were eluted with 25μL nonreducing sample buffer. The Western blot was first probed with anti-Rac1 antibody and then reprobed with anti-HA antibody. HA-tagged GST and Pak1-PBD were immunoprecipitated by anti-HA antibody (right panel, lane 1-3), and active Rac1 was co-immunoprecipitated with HA-Pak1-PBD (left panel, lane 2).

C. Schematic of the Pierce Spin Column



Related Thermo Scientific Products

26180X	HA-Tagged Positive Control, 500 μ L
26181-2	Immobilized Anti-HA agarose, 1mL, 5mL resin
26183	Anti-HA Antibody, 100 μ g
26184	HA Peptide, 5mg
78430	Halt Protease Inhibitor Single-Use Cocktail, 100 μ L $\times$ 24 microtubes
78425	Halt Protease Inhibitor Single-Use Cocktail, EDTA-Free, 100 μ L $\times$ 24 microtubes
24594	GelCode [®] Blue Safe Protein Stain, colloidal coomassie protein stain for SDS-PAGE, 1L
24615	Imperial [™] Protein Stain, coomassie protein stain for SDS-PAGE, 1L
24612	Pierce Silver Stain, silver stain for SDS-PAGE, kit for 20 mini-gels
34080	SuperSignal West Pico Chemiluminescent Substrate for HRP, 500mL
34095	SuperSignal West Femto Chemiluminescent Substrate for HRP, 100mL
69705	Pierce Spin Columns and Accessories, 25 units (see website for listing of all spin column varieties)
21027	Gentle Ag/Ab Elution Buffer, 500mL, pH 6.6
35602	β -mercaptoethanol, 10 $\times$ 1mL
20291	DTT (Dithiothreitol), No-Weigh [™] Format, 7.7mg $\times$ 48 microtubes

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