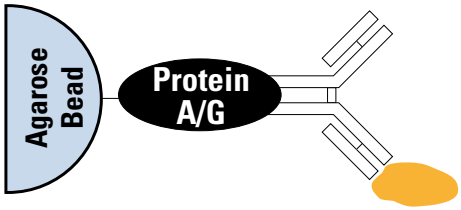
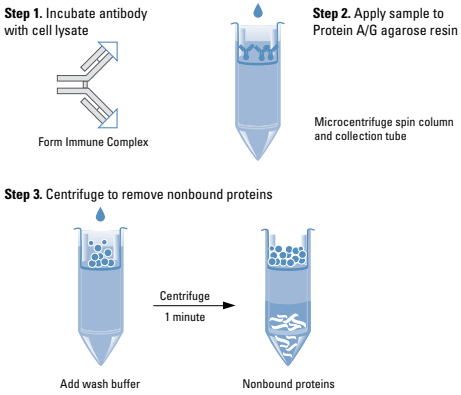
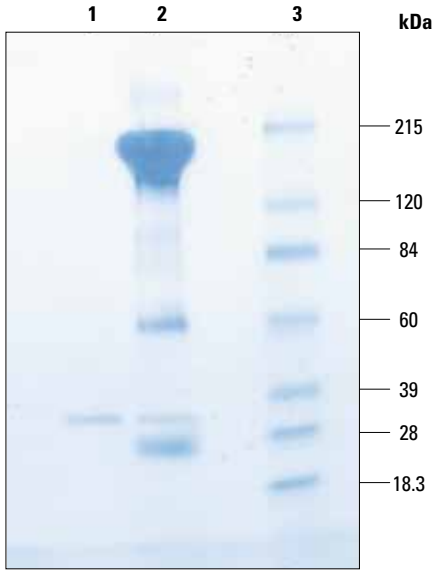


Capture antibody-antigen complexes with Protein A/G agarose beads



The Thermo Scientific Pierce classic IP kit protocol.



Immunoprecipitation with crosslinking enables antigen purification without interference from the IP antibody. *E. coli* cells expressing green fluorescent protein (GFP) were extracted with Thermo Scientific Pierce B-PER Bacterial Protein Extraction Reagent (**PN78248**) and then immunoprecipitated using a polyclonal goat anti-GFP antibody. Eluted IP products were separated by SDS-PAGE and Coomassie stained (**PN24590**) to detect total protein. Lane 1: Single product obtained using the Pierce crosslink IP kit method, Lane 2: Antigen and antibody fragments resulting from traditional IP method and Lane 3: Molecular weight marker.

Immunoprecipitation and co-immunoprecipitation

Immunoprecipitation

Immunoprecipitation (IP) is one of the most widely used methods for antigen purification and detection. The basic principle of an IP is very simple. An antibody (monoclonal or polyclonal) against a specific target antigen is allowed to form an immune complex with that target in a sample, such as a cell lysate. The immune complex is then captured onto a solid support (e.g., beaded agarose) via an immobilised antibody binding protein such as Protein A or Protein G. The process of capturing this complex from the solution is referred to as precipitation. Any proteins not precipitated by the antibody are washed away. Finally, components of the bound immune complex (both antigen and antibody) are eluted from the support and analysed by SDS-PAGE (gel electrophoresis), often followed by Western blot detection to verify the identity of the antigen.

Co-immunoprecipitation

Co-immunoprecipitation is an extension of IP based on the potential of IP reactions to capture and purify not only the primary target (i.e., the antigen) but also whatever other macromolecules are bound to the target by native interactions in the sample solution. Therefore, whether or not an experiment is called an IP or co-IP depends only on whether the focus is the primary target (antigen) or secondary targets (interacting proteins).

Classic immunoprecipitation kit, Thermo Scientific Pierce

Thermo SCIENTIFIC

NEW

PN

For the rapid and efficient recovery of immune-complexed proteins.

- Improves assay consistency - spin columns eliminate resin loss, improve sample recovery and ensure efficient separation from resin
- Immunoprecipitate (IP) target proteins from crude lysates in just four easy steps
- Complete an entire experiment in less than one hour
- Reuse the Protein A/G agarose resin for future IPs
- Kit includes a compatible cell lysis reagent and sufficient components to perform at least 50 IP experiments

The Thermo Scientific Pierce classic IP kit uses microcentrifuge spin columns and collection tubes for easy separation of the Protein A/G agarose resin from the recovered protein.

Catalogue No	Description
PN26146	**Pierce classic IP kit** Sufficient reagents to immobilise several antibodies and perform at least 50 IP reactions. Includes: Protein A/G Plus agarose, 2mL Control agarose resin, 2mL IP lysis/wash buffer, 2 x 50mL Conditioning buffer (100X), 5mL Tris buffered saline (25X), 25mL Elution buffer, 50mL Sample loading buffer (5X), 5mL Spin columns and collection tubes

Crosslinking immunoprecipitation kit, Thermo Scientific Pierce

Thermo SCIENTIFIC

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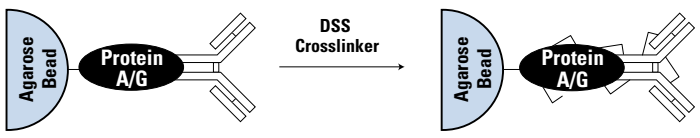
- No elution of antibody heavy and light chains to interfere in SDS-PAGE analysis of purified protein
- Spin columns eliminate resin loss and produce more efficient separation of solutions compared to traditional IP
- Kit includes a compatible cell lysis reagent and sufficient components to immobilise multiple antibodies at a variety of scales and perform at least 50 IP experiments

The Thermo Scientific Pierce crosslink IP kit extends the functionality of traditional immunoprecipitation (IP) methods by adding crosslinking technology and microcentrifuge spin-column sample handling. The primary benefits of these features are target protein purification without contamination by the antibody and highly effectively washes and sample separation from the beaded agarose resin.

The Thermo Scientific Pierce crosslink IP kit method involves capturing the IP antibody to Protein A/G beaded agarose resin and covalently immobilising it to the support by crosslinking with disuccinimidyl suberate (DSS) (see image below). The antibody resin is then incubated with the sample that contains the antigen of interest, allowing the antibody:antigen complex to form. After washing to remove nonbound sample components, the antigen is recovered using the elution buffer supplied in the kit. The entire procedure is performed in a microcentrifuge spin column, allowing solutions to be fully separated from the agarose resin upon brief centrifugation. Only antigen is eluted by the procedure (see Figure), enabling it to be identified and further analysed without interference from antibody fragments.



Immobilised Protein A/G binds antibody | **Disuccinimidyl Suberate Crosslinks proteins** | **Oriented and covalently immobilised antibody**