Immunoprecipitation (IP) is a routinely used immunochemical technique to determine valuable information about a given antigen. The method uses antibody-antigen precipitation to separate a specific antigen from a complex mixture of proteins contained in whole cell lysates or culture supernatants. Applications of immunoprecipitation include: determination of protein molecular weight, protein-protein interactions, biochemical characteristics (enzymatic activity), post-translational modifications and expression levels of a particular antigen. The procedure can be divided into the following steps:

1. Lyse cells to release the antigen and subsequent sample preparation.
2. Incubate specific antibody with sample containing protein (antigen).
3. Immune complex captured with immobilized Protein A or G agarose gel matrix (Protein A or G binds to the antibody, which is bound to the antigen).
4. Wash gel matrix with buffer to remove unbound sample.
5. Elute antigen/antibody complex from the gel by boiling in reducing SDS/PAGE buffer.
6. Analyze immunoprecipitated proteins using gel electrophoresis.

SDS-PAGE analysis of immunoprecipitation methods
A green fluorescent protein (GFP) fusion protein expressed in Escherichia coli was lysed with B-PER* Bacterial Protein Extraction Reagent (PI-78243) and immunoprecipitated using a goat anti-GFP antibody. The antibody was either incubated directly with the bacterial lysate using the classical method or immobilized to the Protein G support provided in the Seize X Bacterial Immunoprecipitation Kit. Immunoprecipitated proteins were reduced, run on an SDS-PAGE gel and stained with GelCode* Blue Stain Reagent (PI-24590). Lane 1 shows a single band from the GFP fusion protein immunoprecipitated using the Seize X Method. Lane 2 shows the classical method in which the immunoprecipitated GFP protein is contaminated with the antibody heavy and light chains. Lane 3 shows BlueRanger* Prestained Protein Molecular Weight Markers (PI-26681).

Pierce* Seize* X Protein G Immunoprecipitation Kit

Includes: ImmunoPure* Immobilized Protein G Plus; Binding/Wash Buffer 1; Quenching/Wash Buffer 2; Elution Buffer; 5X Sample Loading Buffer; DSS; Handee* Spin Cup Columns; Handee Microcentrifuge Tubes

- **No antibody contamination** – antibody heavy and light chains do not appear on SDS-PAGE analysis of precipitated protein
- **Improved assay reliability** – Handee Spin Cup Columns format eliminates resin loss and separates solutions efficiently
- **More economical** – valuable immobilized antibody can be reused to immunoprecipitate more samples
- **Complete kits** – choose kits with or without cell lysis reagents for bacterial, mammalian or yeast cells

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<td>Seize X Protein G</td>
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Pierce® B-PER® Bacterial Protein Extraction Reagents

Recover both soluble and insoluble recombinant proteins from bacterial lysates

**B-PER Bacterial Protein Extraction Reagent**
- Flexible enough to use for any scale of protein extraction
- Does not solubilize inclusion bodies, free of enzymatic components
- Does not require special instrumentation—just add to a bacterial pellet and shake for 10 minutes.
- Uses a patented, mild, nonionic detergent in a Tris-based buffer
- Compatible with GST, 6xHis, and other affinity purifications, also with baculovirus-expressed proteins

**B-PER II Bacterial Protein Extraction Reagent**
- Ideal for small-volume recombinant protein extraction
- Extracts twice the yield of both total soluble protein and recombinant green fluorescent protein after the first round than does original B-PER Reagent (after five rounds, extraction yields are similar)

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<th>Description</th>
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Pierce® M-PER® Mammalian Protein Extraction Reagents

Disrupts mammalian cell membranes at low concentrations without denaturing proteins

- Completes extraction in five minutes at room temperature—no freeze-thaw cycles or sonication required
- Produces 25% more protein than freeze-thaw cycles and 20% more protein than sonication
- Lyses adherent cells without scraping from the culture dish, enabling use of the reagent with 96-well plates
- Suitable for high-throughput cell lysis and subsequent screening assays
- Compatible with downstream reporter assays, kinase assays, immunoassays, and protein assays

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Pierce® ProFound® Co-Immunoprecipitation Kits

Universal systems accommodate any antibody needed for co-immunoprecipitation (co-IP)

Allow for the isolation of native protein complexes from a lysate or other mixture. Combine an amine-reactive gel, reagents for direct covalent immobilization of the primary antibody, reagents for control experiments, buffers for protein binding and recovery, and spin columns to provide consistent, reproducible results and maximum protein recovery.

- Allow immobilization of the antibody-protein complex directly on the matrix without the loss of antibody activity
- Retain the antibody during elution of the co-IP complex, allowing the immobilized antibody support to be reused up to 10 times
- Eliminate the interference from antibody fragments commonly encountered with Protein A or Protein G supports
- Provide control resin options for identification of protein bands that may result from nonspecific interactions of the sample with the base support

Sufficient to immobilize up to 10 antibodies and perform at least 40 co-IP reactions using 25μL coupling gel.

<table>
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<tr>
<th>Description</th>
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