Seize[™] X Immunoprecipitation Kits

Recover more protein without antibody protein band interference.

The Seize™ X Orientated Antibody Advantage

Seize[™] X Kits combine cross-linking and affinity chromatography expertise to offer a new and improved immunoprecipitation method. First, the primary antibody is bound and immobilized to a Protein A or Protein G support using cross-linking agent (DSS). This properly **orients** the antibody to "seize" protein from crude cell lysate applied to the immobilized antibody support (Figure 1). Unbound proteins are then centrifuged away and the protein is recovered by using an elution buffer. Analysis on SDS-PAGE gel shows only a single band for the immunoprecipitated protein without interference from antibody heavy and light chain bands (Figure 2). The immobilized antibody support recovers more protein and can be reused for future samples. The Seize[™] X Kit includes sufficient reagents to immobilize four primary antibodies and complete 10 immunoprecipitations (IPs) with each antibody (i.e., 40 IPs).

The classic immunoprecipitation method incubates the antibody first with cell lysate to form an antibody-antigen complex. This complex is then precipitated with immobilized Protein A or Protein G, centrifuged, and boiled with odorous reducing agents. The proteins assessed by SDS-PAGE show interfering protein bands (heavy and light chains) from the denatured antibody. Both the antibody and the immobilized Protein A or G support are destroyed and cannot be reused. For conserving precious primary antibodies and SDS-PAGE analysis without antibody chain interference, Seize[™] X Immunoprecipitation Kits offer a better alternative.

Features/Benefits:

- No antibody contamination—antibody heavy and light chains do not appear on stained SDS-PAGE analysis of precipitated protein
- **Improved protein recovery**—immunoprecipitate out more target proteins from cell lysates compared to the classic method
- **More economical**—precious primary antibody can be reused to immunoprecipitate more samples
- Complete kits—choose kits with or without cell lysis reagents for bacterial, mammalian or yeast cells

Figure 1. The Seize" X Kit Immunoprecipitation Protocol

Step 1. Cross-link Antibody to Protein G or A Support



Step 2. Lyse Cell and Apply Sample to Antibody Support



Step 3. Wash Away Unbound Proteins



Step 4. Elute Bound Protein



Step 5. Analyze Protein via SDS-PAGE

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Single protein bandno bands from antibody heavy or light chains

Figure 2. 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 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 (Product # 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.

