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Crosslinking Immunoprecipitation Beads

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1 Works for me dx.doi.org/10.17504/protocols.io.bdhki34w

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ABSTRACT

Immunoprecipitation with non-covalent capture of antibodies is a rapid and effective method for enriching samples with desired antigens. Interference by the antibody in down-stream analysis of intact proteins by mass spectrometry can be eliminated by covalent linkage of antibodies to magnetic beads. While several amine-reactive bifunctional linkers have been used for this application, dimethyl pimelimidate (DMP) are particularly advantageous due to its water-solubility, stability of resulting amidines at low pH, and retention of positive charge at reaction sites. By cross-linking to Protein A/G magnetic beads, a greater portion of antibodies are correctly oriented and able to engage their antigen. Cross-linking antibodies to Protein A/G beads with DMP is a quick and effective method that mitigates interference from heavy and light chain IgG in down-stream mass spectrometry applications.

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GUIDELINES

The ratio of beads to antibody in this protocol are a general starting point but may need to be adjusted for each antibody due to variance in the binding affinity of different IgG isoforms to Protein A/G. This protocol has also been tested with Dynabead Protein G beads (Thermo Cat. No. 10003D) with success.

MATERIALS TEXT

MATERIALS

DynaMag™-2 Magnet **Life Technologies Catalog #12321D**

Pierce Protein A/G Magnetic Beads **Thermo Fisher Scientific Catalog #88802**

DMP (dimethyl pimelimidate) **Thermo Fisher Catalog #21667**

Pierce™ Bovine Serum Albumin Standard Ampules, 2 mg/mL **Thermo Fisher Catalog #23209**

Ethanolamine **Millipore Sigma Catalog #15014**

Triethanolamine **Millipore Sigma Catalog #90278**

Glycine **Fisher Scientific Catalog #BP381-500**

Binding Buffer
250 mM Tris-HCl, pH 8.0
TEA Wash Buffer
500 mM triethanolamine, pH 8.9
Crosslinking Buffer
500 mM triethanolamine, pH 8.9, 50 mM DMP
Quenching Buffer
500 mM ethanolamine
Basic Elution Buffer
500 mM triethanolamine, pH 10.5
Acidic Elution Buffer
100 mM glycine, pH 2.5
TBS
20 mM Tris, pH 7.5, 150 mM NaCl
Storage Buffer
200 ug/mL BSA, 20 mM Tris, pH 7.5, 150 NaCl

Complex Formation 1d

1 Form complex with beads and antibody

1.1 Add 900 uL Binding Buffer and 100 uL magnetic beads to 1.5 mL LoBind tube.

1.2 Collect beads with magnet and resuspend with 900 uL Binding Buffer.

DynaMag-2
Magnet
Invitrogen 12321D

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1.3 Add 50 ug IgG antibody.

2 Incubate overnight with rotation.

Tube Rovolver

Fisher 88861051

4 °C Overnight

Crosslink Beads 1h 15m

3 Prepare fresh triethanolamine and ethanolamine buffers. DMP should be added to Crosslinking Buffer immediately before use.

4 Collect beads with magnet and remove supernatant. Wash beads with 1 mL TEA Wash Buffer.

5 Resuspend beads with 1 mL Crosslinking Buffer and incubate at ambient temperature for 1 hr while rotating. 1h

01:00:00 Room temperature

6 Collect beads with magnet and immediately wash beads with 1 mL Quenching Buffer. Resuspend beads in 1 mL Quenching Buffer and incubate at ambient temperature for 15 min while rotating. 15m

00:15:00 Room temperature

7 Collect beads with magnet and wash beads with 1 mL TEA Wash Buffer, 1 mL Basic Elution Buffer, 1 mL Acidic Elution Buffer, and 1 mL TBS twice.

8 Store beads in 1 mL Storage Buffer at 4 °C

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