JAN 30, 2021

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.bdhki34w

Protocol Citation: Bryon Drown, Caroline DeHart, Kelleher KRG KRG Research Group 2021. Crosslinking Immunoprecipitation Beads. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.bdhki34w>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working
 We use this protocol and it works for us. We have evaluated Pierce Protein A/G beads and Dynabead Protein G beads with monoclonal antibodies from mouse and rabbit sources.

Created: Mar 10, 2020

Last Modified: Jan 30, 2021

PROTOCOL integer ID:
 34060

Crosslinking Immunoprecipitation Beads

Bryon Drown¹, Caroline DeHart²,
 Kelleher KRG KRG Research Group²

¹Purdue University; ²Northwestern University

Bryon Drown
 Purdue University

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.

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

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 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #15014**

⊗ Triethanolamine **Merck MilliporeSigma (Sigma-Aldrich) 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.

Equipment

DynaMag-2

NAME

Magnet

TYPE

Invitrogen

BRAND

12321D

SKU

<https://www.thermofisher.com/order/catalog/product/12321D#/12321D>

LINK

1.3 Add 50 ug IgG antibody.

2 Incubate overnight with rotation.

Equipment

Tube Rolver

NAME

Fisher

BRAND

88861051

SKU

<https://www.thermofisher.com/order/catalog/product/88881001#/88881001>

LINK

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. 01:00:00 Room temperature 1h
- 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. 00:15:00 Room temperature 15m
- 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