



Jan 30, 2021

Crosslinking Immunoprecipitation Beads

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Works for me

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



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

DOI

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

PROTOCOL CITATION

Bryon Drown, Caroline DeHart, Kelleher KRG Research Group 2021. Crosslinking Immunoprecipitation Beads. protocols.io

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

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CREATED

Mar 10, 2020

LAST MODIFIED

Jan 30, 2021

PROTOCOL INTEGER ID

34060

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

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.

mprotocols.io 01/30/2021

Citation: Bryon Drown, Caroline DeHart, Kelleher KRG Research Group (01/30/2021). Crosslinking Immunoprecipitation Beads.

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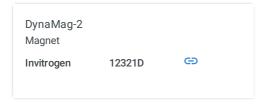
Complex Formation 1d

Form complex with beads and antibody

Storage Buffer

200 ug/mL BSA, 20 mM Tris, pH 7.5, 150 NaCl

- 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.



1	2	٨٨٨	50	ша	Iac	antibody	
- 1	.3	Auu	ΟU	ug	igu	antibody	

2 Incubate overnight with rotation.

Tube Rove	Tube Rovolver								
Fisher	88861051	©							

84°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

- 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 8 4 °C