Cross-linking of IgG to Protein A or G Beads (S1425/S1430)

New England Biolabs

Abstract

This protocol consists of an IgG purification step followed by covalent cross-linking of the IgG to the Protein A/G solid support. For IgG that has been previously purified, proceed directly to the cross-linking protocol.

Citation: New England Biolabs Cross-linking of IgG to Protein A or G Beads (S1425/S1430). **protocols.io** dx.doi.org/10.17504/protocols.io.crdv25
Published: 10 Feb 2015

Guidelines

Overview

Materials Needed:

Protein A (NEB #S1425S) or Protein G (NEB #S1430S) Magnetic Beads
Elution Buffer: 0.1 M glycine-HCl (pH 2.5)
Binding Buffer: 0.1 M NaPhosphate Buffer (pH 8.0)
Dimethyl pimelidate dihydrochloride (Sigma, D-8388) dissolved at 25 mM in Cross-linking Buffer.
Cross-linking Buffer: 0.2 M triethanolamine (pH 8.2)
Blocking Buffer: 0.1 M ethanolamine (pH 8.2)
Immunoglobulin in Binding Buffer

This protocol consists of an IgG purification step followed by covalent cross-linking of the IgG to the Protein A/G solid support. For IgG that has been previously purified, proceed directly to the cross-linking protocol.

Materials

- Protein A Magnetic Beads - 1 ml S1425S by New England Biolabs
- Protein G Magnetic Beads - 1 ml S1430S by New England Biolabs

Protocol

IgG Purification

Step 1.
Vortex and thoroughly resuspend Protein A Magnetic Beads

IgG Purification

Step 2.

Aliquot **100 µl** of bead suspension to a sterile microcentrifuge tube

AMOUNT

100 µl Additional info:

IgG Purification

Step 3.

(wash #1) Add **500 µl** 0.1 M NaPhosphate Buffer (pH 8.0)

AMOUNT

500 µl Additional info:

IgG Purification

Step 4.

(wash #1) Vortex to resuspend

DURATION

00:30:00

IgG Purification

Step 5.

(wash #1) Apply magnet for 30 seconds, to pull beads to the side of the tube

DURATION

00:00:30

IgG Purification

Step 6.

(wash #1) Remove supernatant

IgG Purification

Step 7.

(wash #2) Add **500 µl** 0.1 M NaPhosphate Buffer (pH 8.0)

AMOUNT

500 µl Additional info:

IgG Purification

Step 8.

(wash #2) Vortex to resuspend

IgG Purification

Step 9.

(wash #2) Apply magnet for 30 seconds, to pull beads to the side of the tube

DURATION

00:00:30

IgG Purification

Step 10.

(wash #2) Remove supernatant

IgG Purification

Step 11.

Add to the beads **80 µl** of 0.1 M NaPhosphate Buffer (pH 8.0)

AMOUNT

80 µl Additional info:

IgG Purification

Step 12.

Add 15-25 µ**l** of serum **OR** 20 µg purified IgG in a maximum volume of 30 µl

IgG Purification

Step 13.
Mix thoroughly and incubate at 4°C with agitation for 30 minutes

DURATION
00:30:00

IgG Purification

Step 14.
Apply magnet and remove supernatant.

IgG Purification

Step 15.
(wash #1) Add 500 µl 0.1 M NaPhosphate Buffer (pH 8.0)

IgG Purification

Step 16.
(wash #1) Vortex to resuspend

IgG Purification

Step 17.
(wash #1) Apply magnet for 30 seconds, to pull beads to the side of the tube

DURATION
00:00:30

IgG Purification

Step 18.
(wash #1) Remove supernatant

IgG Purification

Step 19.
(wash #2) Add 500 µl 0.1 M NaPhosphate Buffer (pH 8.0)

IgG Purification

Step 20.
(wash #2) Vortex to resuspend

IgG Purification

Step 21.
(wash #2) Apply magnet for 30 seconds, to pull beads to the side of the tube

DURATION
00:00:30

IgG Purification

Step 22.
(wash #2) Remove supernatant

IgG Purification

Step 23.
(wash #3) Add 500 µl 0.1 M NaPhosphate Buffer (pH 8.0)

IgG Purification

Step 24.
(wash #3) Vortex to resuspend

IgG Purification

Step 25.
(wash #3) Apply magnet for 30 seconds, to pull beads to the side of the tube

DURATION
00:00:30

IgG Purification

Step 26.

(wash #3) Remove supernatant

NOTES

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At this point the purified IgG can be eluted from the beads or used directly for immunoprecipitation of target proteins. The purified IgG can also be cross-linked to the Protein A beads (see cross-linking protocol) to create a reusable immunoprecipitation bead which prevents the co-elution of antibody with target protein.

IgG Cross-linking

Step 27.

Add **1 ml** of Cross-linking Buffer (0.2 M triethanolamine, [pH 8.2]) to the Protein A/G immobilized antibody (wash **a**)

AMOUNT

1 ml Additional info:

NOTES

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At this point the purified IgG can be eluted from the beads or used directly for immunoprecipitation of target proteins. The purified IgG can also be cross-linked to the Protein A beads (see cross-linking protocol) to create a reusable immunoprecipitation bead which prevents the co-elution of antibody with target protein.

IgG Cross-linking

Step 28.

Vortex to resuspend (wash **a**)

IgG Cross-linking

Step 29.

Apply magnet for 30 seconds, to pull beads to the side of the tube (wash **a**)

DURATION

00:00:30

IgG Cross-linking

Step 30.

Remove supernatant (wash **a**)

IgG Cross-linking

Step 31.

Add 1 ml of Cross-linking Buffer (0.2 M triethanolamine, [pH 8.2]) to the Protein A/G immobilized antibody (wash **b**)

IgG Cross-linking

Step 32.

Vortex to resuspend (wash **b**)

IgG Cross-linking

Step 33.

Apply magnet for 30 seconds, to pull beads to the side of the tube (wash **b**)

DURATION

00:00:30

IgG Cross-linking

Step 34.

Remove supernatant (wash **b**)

IgG Cross-linking

Step 35.

Resuspend in 1 ml Cross-linking Buffer containing 25 mM DMP (6.5 mg DMP/ml of buffer)

IgG Cross-linking

Step 36.

Mix thoroughly and incubate at room temperature for 45 minutes with agitation

DURATION

00:45:00

IgG Cross-linking

Step 37.

Apply magnet for 30 seconds, to pull beads to the side of the tube

DURATION

00:00:30

IgG Cross-linking

Step 38.

Remove supernatant

IgG Cross-linking

Step 39.

Add 1 ml Blocking Buffer (0.1 M ethanolamine, [pH 8.2])

IgG Cross-linking

Step 40.

Vortex to resuspend

IgG Cross-linking

Step 41.

Apply magnet for 30 seconds, to pull beads to the side of the tube

DURATION

00:00:30

IgG Cross-linking

Step 42.

Remove supernatant

IgG Cross-linking

Step 43.

Add 1 ml of Blocking Buffer

AMOUNT

1 ml Additional info:

IgG Cross-linking

Step 44.

Vortex to resuspend

IgG Cross-linking

Step 45.

Incubate for 1 hour at room temperature with agitation

DURATION

01:00:00

IgG Cross-linking

Step 46.

Apply magnet for 30 seconds, to pull beads to the side of the tube

DURATION

00:00:30

IgG Cross-linking

Step 47.

Remove supernatant

IgG Cross-linking

Step 48.

(wash #1) Add 1 ml of PBS

AMOUNT

1 ml Additional info:

IgG Cross-linking

Step 49.

(wash #1) Vortex to resuspend

AMOUNT

1 ml Additional info:

IgG Cross-linking

Step 50.

(wash #1) Apply magnet for 30 seconds, to pull beads to the side of the tube

DURATION

00:00:30

IgG Cross-linking

Step 51.

(wash #1) Remove supernatant

IgG Cross-linking

Step 52.

(wash #2) Add 1 ml of PBS

IgG Cross-linking

Step 53.

(wash #2) Vortex to resuspend

IgG Cross-linking

Step 54.

(wash #2) Apply magnet for 30 seconds, to pull beads to the side of the tube

DURATION

00:00:30

IgG Cross-linking

Step 55.

(wash #2) Remove supernatant

IgG Cross-linking

Step 56.

(wash #3) Add 1 ml of PBS

IgG Cross-linking

Step 57.

(wash #3) Vortex to resuspend

IgG Cross-linking

Step 58.

(wash #3) Apply magnet for 30 seconds, to pull beads to the side of the tube

DURATION

00:00:30

IgG Cross-linking

Step 59.

(wash #3) Remove supernatant

IgG Cross-linking

Step 60.

Add 1 ml Elution Buffer (0.1 M glycine-HCl [pH 2.5])

AMOUNT

1 ml Additional info:

IgG Cross-linking

Step 61.

Vortex to resuspend

IgG Cross-linking

Step 62.

Apply magnet for 30 seconds, to pull beads to the side of the tube

DURATION

00:00:30

NOTES

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This elutes bound antibody that is not cross-linked with DMP.

IgG Cross-linking

Step 63.

Remove supernatant

IgG Cross-linking

Step 64.

Resuspend and store beads in 100 µl PBS, 0.1% Tween 20, 0.02% sodium azide