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Feb 23, 2022

Cross-linking of IgG to Protein A or G Beads (S1425/S1430) V.2

New England Biolabs[1]

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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.

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Protein A, Protein G, cross-linkage, cross linked, covalent cross-linking of the IgG to protein A/G, igG purification to beads

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Overview

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 **New England Biolabs Catalog #S1425S**

Protein G Magnetic Beads - 1 ml **New England Biolabs Catalog #S1430S**

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)
- 10x Phosphate Buffered Saline **Gibco, ThermoFisher Catalog #70011044**
- Tween 20 **Sigma Aldrich Catalog #P9416-50ML**
- Sodium azide **Sigma Aldrich Catalog #71289**
- Immunoglobulin in Binding Buffer
- 100 µl PBS, 0.1% Tween 20, 0.02% sodium azide

For hazard information and safety warnings, please refer to the SDS (Safety Data Sheet).

The IgG Purification protocol is for the binding of **20 µg purified IgG** or isolation of **20 µg IgG from serum** .

IgG Immobilization

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The IgG immobilization protocol is for the binding of **20 µg purified IgG** or isolation of **20 µg IgG from serum** .

Vortex and thoroughly resuspend Protein A Magnetic Beads.

2

Aliquot **100 µL bead suspension** to a sterile microcentrifuge tube.

3

Add **500 µL 0.1 M NaPhosphate Buffer (pH 8.0)** and vortex to resuspend. Apply magnet for **00:00:30** , to pull beads to the side of the tube and remove supernatant. (Wash 1/2)

4

Repeat wash: Add **500 µL 0.1 M NaPhosphate Buffer (pH 8.0)** and vortex to resuspend. Apply magnet for **00:00:30** , to pull beads to the side of the tube and remove supernatant. (Wash 2/2)

5

Add to the beads **80 µL 0.1 M NaPhosphate Buffer (pH 8.0)** .

6

Add **15 µL** - **25 µL serum OR 20 µg purified IgG** in a maximum volume of **30 µL** .

7

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Mix thoroughly and incubate at **4 °C** with agitation for **00:30:00** .

8 Apply magnet and remove supernatant.

9

Add **500 µL 0.1 M NaPhosphate Buffer (pH 8.0)** and vortex to resuspend. Apply magnet for **00:00:30** , to pull beads to the side of the tube and remove supernatant. (Wash 1/3)

10

Add **500 µL 0.1 M NaPhosphate Buffer (pH 8.0)** and vortex to resuspend. Apply magnet for **00:00:30** , to pull beads to the side of the tube and remove supernatant. (Wash 2/3)

11

Add **500 µL 0.1 M NaPhosphate Buffer (pH 8.0)** and vortex to resuspend. Apply magnet for **00:00:30** , to pull beads to the side of the tube and remove supernatant. (Wash 3/3)

IgG Cross-linking to Protein A/G Magnetic Beads 30m

12

Add **1 mL Cross-linking Buffer (0.2 M triethanolamine, [pH 8.2])** to the Protein A/G immobilized antibody.(Wash 1/2)

> 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.

13

Vortex to resuspend. (Wash 1/2)

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14 Apply magnet for **00:00:30** , to pull beads to the side of the tube and remove supernatant. (Wash 1/2)

15 

Add **1 mL Cross-linking Buffer (0.2 M triethanolamine, [pH 8.2])** to the Protein A/G immobilized antibody.(Wash 2/2)

16 

Vortex to resuspend. (Wash 2/2)

17 Apply magnet for **00:00:30** , to pull beads to the side of the tube and remove supernatant. (Wash 2/2)

18 

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

19 

Mix thoroughly and incubate at **Room temperature** for **00:45:00** with agitation.

20 Apply magnet for **00:00:30** , to pull beads to the side of the tube and remove supernatant.

21 

Add **1 mL Blocking Buffer (0.1 M ethanolamine, [pH 8.2])** .

22 

Vortex to resuspend.

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23 Apply magnet for **00:00:30** , to pull beads to the side of the tube and remove supernatant.

24 

Add **1 mL Blocking Buffer** .

25 

Vortex to resuspend.

30m

26 

Incubate for **00:30:00** at **Room temperature** with agitation.

27 Apply magnet for **00:00:30** , to pull beads to the side of the tube and remove supernatant.

28 

Add **1 mL PBS** . (Wash 1/3)

29 

Vortex to resuspend. (Wash 1/3)

30 Apply magnet for **00:00:30** , to pull beads to the side of the tube and remove supernatant. (Wash 1/3)

31 

Add **1 mL PBS** . (Wash 2/3)

32 

Vortex to resuspend. (Wash 2/3)

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33 Apply magnet for **00:00:30** , to pull beads to the side of the tube and remove supernatant. (Wash 2/3)

34 

Add **1 mL PBS** . (Wash 3/3)

35 

Vortex to resuspend. (Wash 3/3)

36 Apply magnet for **00:00:30** , to pull beads to the side of the tube and remove supernatant. (Wash 3/3)

37 

Add **1 mL Elution Buffer (0.1 M glycine-HCl [pH 2.5])** .

38 

Vortex to resuspend.

39 Apply magnet for **00:00:30** , to pull beads to the side of the tube and remove supernatant.

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

40 

Add **1 mL PBS** . (1/2)

41 

Vortex to resuspend. (1/2)

42 Apply magnet for **00:00:30** , to pull beads to the side of the tube and remove supernatant. 30s

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(1/2)

43 

Add **1 mL PBS** . (2/2)

44 

Vortex to resuspend. (2/2)

45 Apply magnet for **00:00:30** , to pull beads to the side of the tube and remove supernatant. (2/2)

30s

46 

Resuspend and store beads in **100 µL PBS** , 0.1% Tween 20, 0.02% sodium azide.

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