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Rapid Purification of IgG Antibodies Kit

Introduction

The Rapid Purification of IgG Antibodies kit is designed for rapid, single-step purification of high-quality IgG from saliva, ascites, serum and tissue culture media or hybridoma supernatants based on protein A/G magnetic beads. In addition, the magnetic beads can be used to immunoprecipitate target proteins from crude cell lysates using a selected primary antibody. The beads are stable and leak resistant over a wide pH range.

Components

Contents \ Cat. No.	MA 101	MA 102	MA 103	MA 104	MA 105	MA 106
BcMag. A/G Beads	1.0ml	2.0ml	3.0ml	2.0ml	5.0ml	10ml
5x Protein A/G Binding /Washing Buffer	12ml	24ml	36ml	X	X	X
1x Protein A/G Elution Buffer	1.0ml	2.0ml	3.0ml	X	X	X
1x Protein A/G Neutralization Buffer	1.0ml	2.0ml	3.0ml	X	X	X

Buffer Composition

- BcMag.Protein A/G Beads (Suspended in 10 mM Tris, 0.15 M NaCl, 0.1% BSA,1 mM EDTA, pH 7.4, 0.1% NaN_3)
- 1x Protein A/G Binding/Washing Buffer (57.7 mM Na_2HPO_4,42.3 mM NaH_2PO_4, pH 7.0)
- 1x Protein A/G Elution Buffer (0.2 M Glycine/HCl, pH 2.5)
- 1x Protein A/G Neutralization Buffer (1.0 M Tris-HCl, pH 9.0)

Binding capacity: ~ 1.0 mg IgG antibodies /ml magnetic beads

Storage: 4^o C (Do not freeze)

Protocol

Note:

1. This protocol is optimized for purifying most IgG antibodies from different sources. However, it is impossible to design a universal kit for all IgG purification because no two antibodies are exactly alike. In order to obtain the best results, each user must determine the optimal working conditions for purification of individual antibodies, especially for those weakly-binding antibodies (see Table 1), based on suggestions in the Troubleshooting section.

2. To ensure optimal binding conditions involving ionic strength and pH, it is necessary to dilute serum samples, ascites fluid or tissue culture at least 1:1 with Binding/Washing buffer prior to the purification. Remove any insoluble materials in the sample by centrifugation or filtration through a 0.2 µm filter.

3. BcMag Protein A/G Beads can not be used to purify IgG antibody from tissue culture supernatants containing fe tal calf serum, because the beads have very high affinity for the bovine IgGs in serum.

4. Prior to purifying IgG, the user should equilibrate all the reagents contained in the kit to room temperature and make 1x working solutions by diluting 5x stock solutions with 4 volumes of double distilled H_2O.

A. Purification

1. Gently shake the bottle containing BcMag.Protein A/G Beads until the magnetic beads are completely suspended. Aliquot an appropriate amount of the beads to a fresh tube.

Note: The optimal amount of beads to be used should be empirically determined by each user based on the amount of the IgG in the crude sample. Too many magnetic beads will result in a higher background; too little will reduce the yield. We recommend 100 µl of the completely suspended beads per 100 µg of IgG antibodies. Usually a high-titer rabbit antiserum has roughly 5 mg/ml of IgG, mouse ascites has roughly 10 mg/ml of IgG, and goat or sheep antiserum has roughly 20 mg/ml of IgG.

2. Place the tube in a magnetic separator and wait for 2-3 min until supernatant becomes clear. Discard all the supernatant. Remove the tube from the magnetic separator and re-suspend the beads with 5 volumes of 1x Binding/Washing Buffer.

Note: Magnetic separators are commercially available from Bioclone Inc.:BcMag separator-2 for holding two individual 1.5 ml centrifuge tubes, Cat. No. MS-01; BcMag separator-6 for holding six individual 1.5 ml centrifuge tubes, Cat. No. MS-02; BcMag separator-24 for holding twenty-four individual 1.5 ml centrifuge tubes, Cat. No. MS-03; BcMag separator-50 for holding one 50 ml and one 15 ml centrifuge tube, Cat. No. MS-04.

3. Place the tube in the magnetic separator, wait for 2-3 min until supernatant becomes clear and discard all the supernatant. Remove the tube from the magnetic separator and re-suspend the beads by adding appropriate amount of antibody sample/ Binding Buffer solution (Mix crude or diluted antibody sample with 1x Binding/Washing Buffer at ratio of 1:2).

4. Mix well by gently pipetting several times with 1x Binding/ Washing Buffer and incubate at 4^o C for 30-45 min with rotational mixing.

5. Wash the beads as in step 2 until the absorbance of elute at 280 nm approaches background level (OD 280 < 0.05).

6. Add an appropriate amount of Elution Buffer to elute the IgG from the magnetic beads. Mix well by gently pipetting several times and incubate at 4^o C for 10 min with rotational mixing. Place the tube in the magnetic separator for 1 min and carefully remove the antibody-containing supernatant into a clean tube.

7. Immediately neutralize the eluted antibody solution by adding 0.1 ml neutralization buffer for each 1.0 ml supernatant and mix well.

8. Desalt and concentrate the eluted fraction by dialysis, gel filtration chromatography or other means.

B. Reusable immunoprecipitation bead preparation

Specific antibodies can be chemically cross-linked to the BcMag Protein A/G beads to create reusable immunoprecipitation beads, avoiding co-elution of antibody with target protein (antigen). Antibody-protein A/G cross-linked magnetic beads can efficiently isolate highly pure target antigen in 2 - 3 hr.

Table 1. Predicted affinity of various IgG classes for Protein A/G binding

IgG (Origin)	Binding (Protein A/G)	IgG (Origin)	Binding (Protein A/G)	IgG (Origin)	Binding (Protein A/G)
Human IgG	S	Chicken IgY	N	Cat IgG	S
Human IgG1	S	Mouse IgG	S	Horse IgG	S
Human IgG2	S	Mouse IgG1	W/S	Horse IgG(c)	W/S
Human IgG3	S	Mouse IgG2a	S	Horse IgG(T)	W
Human IgG4	S	Mouse IgG2b	S	Pig IgG	S
Human IgM	W	Mouse IgG3	S	Dog IgG	S
Human IgA	W	Goat IgG	S	Sheep IgG	S
Human IgA1	S	Rabbit IgG	S	Rat IgG	W/S
Human IgA2	S	Cow IgG	S	Rat IgG2a	S
Human Ig	W	Monkey IgG	S	Rat IgG2b	W/S
Human IgE	W/S	Guinea pig IgG	S	Rat IgG2c	W

S = Strong; W/S = Medium; W = Weak; N = negative

Materials to be supplied by user:

- Cross-linking Buffer: 0.2 M Triethanolamine, pH 8.2
- Block Buffer: 0.1 Methanolamine, pH 8.2
- Washing Buffer: 0.1 M Glycine-HCl, pH 5.2
- Storage Buffer: 1x PBS Buffer, pH 7.5, 0.1% Tween20, 0.02% NaN_3
- DMP: Dimethy pimelidate dihydrochloride (Sigma, Cat No. D-8388)
- PBS Buffer: 137 mM NaCl, 2.7 mM KCl, 4.3 mM, Na_2HPO_4, 1.4 mM KH_2PO_4, pH 7.5

Preparation:

1. Follow the step 5 as described in purification section, add 1.0 ml Cross-linking buffer and pipette several times. Place the tube in the magnetic separator and wait for 1 min. Remove supernatant completely and discard.

2. Add 1.0 ml Cross-linking Buffer containing 25mM DMP. Remove the tube from the magnetic separator. Mix well and gently shake for 45 min at room temperature.

3. Put the tube into the magnetic separator and wait for 1 min. Remove supernatant completely and discard.

4. Add 1.0 ml Block Buffer, remove the tube from the magnetic separator. Mix well, put the tube back into the magnetic separator and wait for 1 min. Completely remove and discard supernatant.

5. Add 1.0 ml Block Buffer. Remove the tube from the magnetic separator. Mix well and gently shake for 1 hr at room temperature.

6. Put the tube back into the magnetic separator and wait for 1 min. Completely remove and discard supernatant.

7. Add 1.0 ml PBS Buffer. Remove the tube from the magnetic separator. Mix well by gently pipetting. Then put the tube back into the magnetic separator and wait for 1 min. Completely remove and discard supernatant.

8. Repeat step 7 twice.

9. Add 1.0 ml washing Buffer. Remove the tube from the magnetic separator. Mix well by gently pipetting. Then put the tube back into the magnetic separator. Wait for 1 min. Completely remove and discard supernatant.

10. Suspend the beads in 100 ul Storage Buffer.

C. BcMag.Protein A/G. beads storage

The beads should be stored in PBS Buffer, 0.1% Tween-20, 0.02% NaN_3, pH 7.2 - 7.5 at 4^o C.

Questions and Answers:

Sometimes, why can not IgG be eluted from the magnetic beads?

- The pH of the Elution Buffer may be incorrect. The orrect pH should be 2.5.
- The elution conditions are too mild to elute the antibody because a few antibodies can only be eluted at pH 2.0.

What accounts for lost or decreased immuno-reactivity of the eluted antibody?

It will not influence the immuno-reactivity for most antibodies once the eluted fraction is immediately neutralized by addition of Neutralization Buffer. However, a few antibodies (e.g., some monoclonal antibodies) are acid-labile and they can irreversibly lose their activity at very low pH values. For those low pH sensitive-antibodies, the user should try other alternative elution methods such as a high salt Elution Buffer (Pierce, Cat.No. 21031).

Why are multiple bands observed in the eluted antibody solution?

Some host proteins may nonspecifically interact with your target antibody. User can add NaCl (50-200mM, final concentration) in the binding and elution buffers.