LifeTein Protein A Magnetic Beads

Introduction

LifeTein Protein A Magnetic Beads are uniform, monodisperse, silica-based superparamagnetic beads covalently conjugated with ultrapure (Purity>97%) recombinant protein A on the surface. The Beads are specifically designed, tested and quality controlled for use of immunoprecipitation, cell sorting when a selected primary antibody is used. The beads are also widely used for quick and efficient one-step purification of IgG antibodies from Serum samples, ascites fluid, plasma or tissue culture supernatant from several species. The binding affinity and specificity are summarized in Table 1.

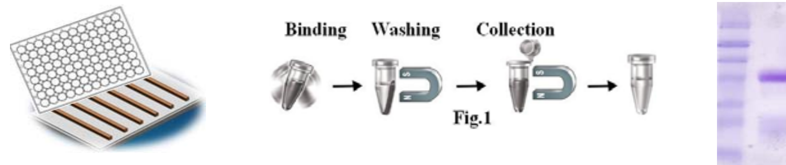

Fig.1

Feature and Benefits

- Quick, Easy and one-step high-throughput procedures; eliminate columns or filters, or laborious repeat pipetting or centrifugation (Fig.1)
- Ultra low non-specific binding due to beads' hydrophilic surface
- High binding capacity
- Scalable - easily adjusts for sample size and automation.

Table 1

Species	Antibody	Binding (Protein A)	Species	Antibody	Binding (Protein A)
Mouse	IgG 1	++	**Sheep**	IgG1	++
	IgG 3	++++		IgG2	++++
	IgG 2a	++++		Total IgG	++
	IgG 2b	++++	**Horse**	IgG(ab)	++
	IgM	-		IgG(c)	++
	Total IgG	++++		IgG(T)	-
Human	IgG1	++++		Total IgG	++
	IgG2	++++	**Goat**	IgG1	++
	IgG3	++		IgG2	++++
	IgG4	++++		Total IgG	++
	IgA	++	**Cow**	IgG1	++
	IgD	-		IgG2	++++
	IgM	++		Total IgG	++
	Fab	++	**Rabbit**	Total IgG	++++
	scFv	++	**Guinea Pig**	Total IgG	++++
	Total IgG	++++	**Pig**	Total IgG	++++
Rat	IgG 1	++	**Cat**	Total IgG	++++
	IgG 2a	-	**Dog**	Total IgG	++++
	IgG 2b	-	**++++ (Strong Binding); +++ (Medium Binding); ++ (Weak Binding); - (No Binding); N/A (No Information)**		
	IgG 2c	++++			
	Total IgG	++			

Product Specificities	
Composition	Silica-coated iron oxide Protein A
Beads Size	~ 1 µm diameter
Number of Beads	~1.7 x 10^8 beads
Magnetization	~40 EMU/g
Type of Magnetization	Superparamagnetic
Effective Density	2.5 g/ml
Stability	Short term: pH 3-11; Long term: pH 4-10
Binding Capacity	~ 1 mg IgG / ml of Beads
Storage	Store at 4°C upon receipt

Buffer Composition

- Protein A Beads (Suspended in 10 mM Tris, 0.15 M NaCl, 0.1% BSA,1 mM EDTA, pH 7.4, 0.1% NaN3)
- 1x Protein A Binding/Washing Buffer (57.7 mM Na2HPO4,42.3 mM NaH2PO4, pH 7.0)
- 1x Protein A Elution Buffer (0.2 M Glycine/HCl,pH 2.5)
- 1x Protein A Neutralization Buffer (1.0 M Tris-HCl, pH 9.0)

Materials Required

- **Magnetic Separator (Not included):**

Protocol

The protocol can be properly scaled up or down.

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 µ m filter.
3. Prior to purifying IgG, the user should equilibrate all the reagents to room temperature.

A. Purification

1. Gently shake the bottle containing beads. Protein A Beads until the magnetic beads are completely suspended. Transfer 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 µ l 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 on a magnetic separator for 1minute. Remove the supernatant while the tube remains on the separator. Remove the tube from the separator and wash the beads with 5 bead volumes of 1x Binding/Washing Buffer by vortex. Place the tube on the magnetic separator for 1 minute. Remove the supernatant while the tube remains on the separator.
3. Repeat step 2 twice.
4. 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). Mix well by gently pipetting several times and incubate at room temperature for 10-20 minutes or 4º C for 30-45 minutes with rotational mixing.
5. Wash the beads as in step 2 until the absorbance of wash at 280 nm approaches background level (OD 280 < 0.05).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 4º C for 10 minutes with rotational mixing. Place the tube in the magnetic separator for 1 minute and carefully remove the antibody-containing supernatant into a clean tube. Immediately neutralize the eluted antibody solution by adding 0.1 ml neutralization buffer for each 1.0 ml supernatant and mix well.
6. Desalt and concentrate the eluted fraction by dialysis, gel filtration chromatography or other methods.

B. Reusable immunoprecipitation bead preparation

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

Materials to be supplied by user:

- Cross-linking Buffer: 0.2 M Triethanolamine, pH 8.2 (Sigma, Cat# T1377)
- Block Buffer: 1 M ethanolamine, pH 8.2 (Sigma , Cat#: 411000)
- 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: Dimethyl pimelinediimidate dihydrochloride (Sigma, Cat No. D8388)
- 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 6 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 minute. Remove supernatant completely...
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 minutes at room temperature.
3. Place the tube on the magnetic separator and wait for 1 minute. Remove supernatant completely
4. Add 1.0 ml Block Buffer, and remove the tube from the magnetic separator. Mix well, put the tube back into the magnetic separator and wait for 1 minute. 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 1minute. Completely remove 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 minute. 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 minute. Completely remove and discard supernatant.
10. Resuspend the beads in Storage Buffer.

C. Beads storage

The beads should be stored in PBS Buffer, 0.1% Tween-20, 0.02% NaN3, pH 7.2 - 7.5 at 4º C.

Questions and Answers:

1. **Sometimes, why IgG could not be eluted from the magnetic beads?**
   - The pH of the Elution Buffer may be incorrect. The correct 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.
2. **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. #: 21031).
3. **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

D. General Affinity Purification Protocol

Note:

This protocol is a general affinity purification procedure. It is impossible to design an universal protocol for all protein purification because no two protein are exactly alike. In order to obtain the best results, each user must determine the optimal working conditions for purification of individual target protein.

1. Transfer optimal amount of the beads to a centrifuge tube. Place the tube on the magnetic separator for 1-3 minutes. Remove the supernatant while the tube remains on the separator.

Note:

It is strongly recommended that a titration be performed to optimize the quantity of beads used for each individual application based on the amount of the target protein in crude sample. Too many magnetic beads used will cause higher backgrounds, while too little beads used will cause lower yields. Each mg of conjugated magnetic beads normally bind to 1-20 µg target protein.

2. Remove the tube and resuspend the beads with 5 bead volume of PBS buffer by vortex for 30 seconds. Leave the tube at room temperature for 1-3 minutes. Place the tube on the magnetic separator for 1-3 minutes. Remove the supernatant while the tube remains on the separator.
3. Repeat step 2 two times
4. Add washed beads to crude sample containing target protein and incubate at room temperature or desired temperature for 1-2 hours (Lower temperature require longer incubation time).
5. Extensively wash the beads with 5 bead volumes of PBS buffer or 1M NaCl until the absorbance of elute at 280 nm approaches background level (OD 280 < 0.05).
6. Elute the target protein by appropriated methods such as low pH (2-4), high pH (10-12), high salt, high temperature, affinity elution or boiling in SDS-PAGE sample buffer.