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A Geno Technology, Inc. (USA) brand name

Protein A Magnetic Beads

(Cat. # 786-902, 786-903)

INTRODUCTION

G-Biosciences' Protein A Magnetic Beads are designed as a rapid and simple tool for immunoprecipitation, purification and/or depletion assays, and other magnetic separation applications. Antibodies easily bind to the Protein A Magnetic Beads due to their high affinity for protein A. The target protein is captured by the antibody:Protein A Magnetic Beads and are temporarily immobilized at the tube wall by magnetic attraction and all other proteins are easily removed.

ITEMS SUPPLIED

Cat. #	Description	Size
786-902	Protein A Magnetic Beads	1ml
786-903	Protein A Magnetic Beads	5 x 1ml

STORAGE CONDITIONS

The beads are shipped at ambient temperature. Upon arrival, store the beads at 4°C. If stored and handled correctly the beads have a 1 year shelf life.

SPECIFICATIONS

Fe_3O_4 beads coated with dextran of an average 1µm in diameter. Protein A is coupled covalently to dextran. Supplied in phosphate buffered saline, pH 7.4, containing 0.02% Tween 20 and 0.09% sodium azide.

PRECAUTIONS

- Do not freeze the magnetic beads
- Do not store near magnetic sources

BINDING PROPERTIES

The binding capacity of Protein A Magnetic Beads is more than 26µg human IgG per 100µl. The binding strength of Protein A Magnetic Beads to different immunoglobulins is listed as below

Species	Antibody Class	Protein A	Protein G
Mouse	Total IgG	++++	++++
	IgG_1	+	+++
	IgG_{2a}	++++	++++
	IgG_{2b}	++++	++++
	IgG_3	+++	+++
Human	Total IgG	++++	++++
	IgG_1	++++	++++
	IgG_2	++++	++++
	IgG_3	+	++++
	IgG_4	++++	++++
Rat	Total IgG	+	++
	IgG_1	-	+
	IgG_{2a}	-	++++
	IgG_{2b}	-	++
	IgG_{2c}	++	+++
Hamster	Total IgG	++	++
Guinea Pig	Total IgG	++++	++
Rabbit	Total IgG	++++	+++
Horse	Total IgG	++	++++
Cow	Total IgG	++	++++
Pig	Total IgG	+++	++
Sheep	Total IgG	+	++
Goat	Total IgG	+	++
Chicken	Total IgG	-	-

Table 1: Relative affinity of Protein A and Protein G for Immunoglobulins

ADDITIONAL ITEMS REQUIRED

- Washing Buffer: 1X PBS buffer (pH 7.4) with0.02% Tween 20
- Elution Buffer: 100mM glycine, pH 3.0
- Neutralization Buffer: 1M Tris-HCl, pH 8.5
- Storage Buffer: 0.01M NaH_2PO_4, 0.15M NaCl, 2.7mM KCl, pH 7.4, 20% ethanol
- Magnetic Stand or magnet

PREPARATION BEFORE USE

Sample preparation: We recommend that for optimal binding the serum samples/ascites fluid or tissue culture media be the addition of $1/10^{th}$ volume of 1.0M Tris, pH 8.0.

PROTOCOL

Preparation of Protein A Magnetic Beads

1. Resuspend the Protein A Magnetic Beads thoroughly by pipetting or vortexing the vial for 15 seconds.
2. Transfer adequate amount of Protein A Magnetic Beads into a clean tube.
   NOTE: *Take appropriate amount of beads according to the binding capacity mentioned above. For example, if 2-3µg of antibody is used in immunoprecipitation test, 5-10µl of Protein A Magnetic Beads is enough for one test. Excess amount of beads may cause high background in some cases.*
3. Place the tube on the magnetic stand for 30-60 seconds to immobilize the beads at tube wall.
4. Discard the supernatant by aspiration with a pipette.
5. Remove the tube from the magnetic stand.
6. Add 200µl Washing Buffer and resuspend the beads by pipetting.
7. Place the tube on the magnetic stand for 30-60 seconds to immobilize the beads at tube wall.
8. Discard the supernatant, and then remove the tube from the magnetic stand.
9. Repeat steps 6-8 twice.
   NOTE: *Protein A Magnetic Beads contains 0.09% NaN_3, so we strongly recommend washing the beads at least three times before use.*

Binding of Antibody

10. Mix appropriate amount of antibody in 200µl Washing Buffer and transfer to the tube from step 9 and vortex for 10 seconds.
11. Incubate for 30 minutes at room temperature with gentle rocking or shaking.
12. Place the tube on the magnetic stand for 30-60 seconds to immobilize the beads at tube wall.
13. Discard the supernatant, and then remove the tube from the magnetic stand.
14. Add 200µl Washing Buffer and resuspend the beads by pipetting.

15. Place the tube on the magnetic stand for 30-60 seconds to immobilize the beads at tube wall.
16. Discard the supernatant, and then remove the tube from the magnetic stand.
17. Repeat steps 14-16 two more times to remove unbound antibody.

IMMUNOPRECIPITATION METHOD

1. Add at least 100µl cell lysate sample containing target antigen to the tube from step 17 and vortex for 10 seconds.
2. Incubate for 30 minutes at room temperature or 4°C overnight with gentle rocking or shaking.
3. Place the tube on the magnetic stand for 30-60 seconds to immobilize the beads at tube wall.
4. Discard the supernatant, and then remove the tube from the magnetic stand.
5. Add 200µl Washing Buffer and resuspend the beads by pipetting.
6. Place the tube on the magnetic stand for 30-60 seconds to immobilize the beads at tube wall.
7. Discard the supernatant, and then remove the tube from the magnetic stand.
8. Repeat steps 5-7 two more times to remove unbound antigen.
9. For SDS-PAGE and Western blot analysis, add appropriate volume of SDS-PAGE Loading Buffer and heat the beads at 95°C for 5 minutes. Load the bead/ loading buffer mix directly onto an SDS-PAGE gel and proceed with electrophoresis and blotting as normal.

ELUTION OF ANTIBODY & ANTIGEN

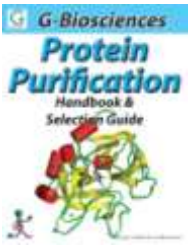
1. Add 20µl Elution Buffer to the bead:antibody:antigen mix and resuspend by pipetting.
2. Incubate with tilt rotation for 2 minutes at room temperature.
3. Place the tube on the magnet stand for 30-60 seconds.
4. Collect the supernatant to a clean tube, and then adjust the pH by adding 2µl Neutralization Buffer (e.g. 1M Tris-HCl, pH 8.5).

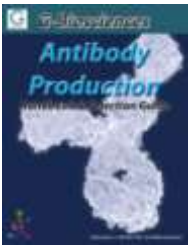
TROUBLESHOOTING

Issue	Suggested Reason	Possible Solution
Immunoglobin binding is low.	Improper resuspension & mixing	Make sure the beads are suspended thoroughly before use
		Mix beads and sample thoroughly and continuously with either a tilt rotation device or a vortexer.
	Protein A has low binding affinity for antibody	Refer to Table 1 to match the binding preference of protein A with various Immunoglobins
	Incubation time and temperature not ideal	Incubation time and temperature can be optimized depending on the sample column and affinity of antibody for target antigen
Non-specific and background binding is high.	Excess of beads used	Reduce the usage amount of beads per test according to the binding capacity
	Insufficient washing	After incubate bead:antibody complex with antigen, increase wash procedures to >5 times.
	Strong non-specific interactions	Increase the concentration of Tween 20 to 0.1% in Washing Buffer prior to elute the sample
	Cross contamination	Ensure all the Washing Buffer is removed

RELATED PRODUCTS

Download our Protein Purification and Antibody Production Handbooks.





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