Magne™ Protein A Beads and Magne™ Protein G Beads for Antibody Purification

INSTRUCTIONS FOR USE OF PRODUCTS G7471, G7472, G7473, G8781, G8782 AND G8783.

Quick PROTOCOL

Antibody Purification Protocol for 50µl Sample Size

Equilibrate Beads

1. Gently vortex or invert the beads to obtain a uniform suspension. Keep the suspension uniform when aliquotting beads.
2. Add 50µl of bead slurry to a 1.5ml microcentrifuge tube. Place in the magnetic stand for 10 seconds.
3. Remove and discard the storage buffer.

Bind

4. Add 500µl of bind/wash buffer. Mix and place in the magnetic stand for 10 seconds. Remove and discard the bind/wash buffer.

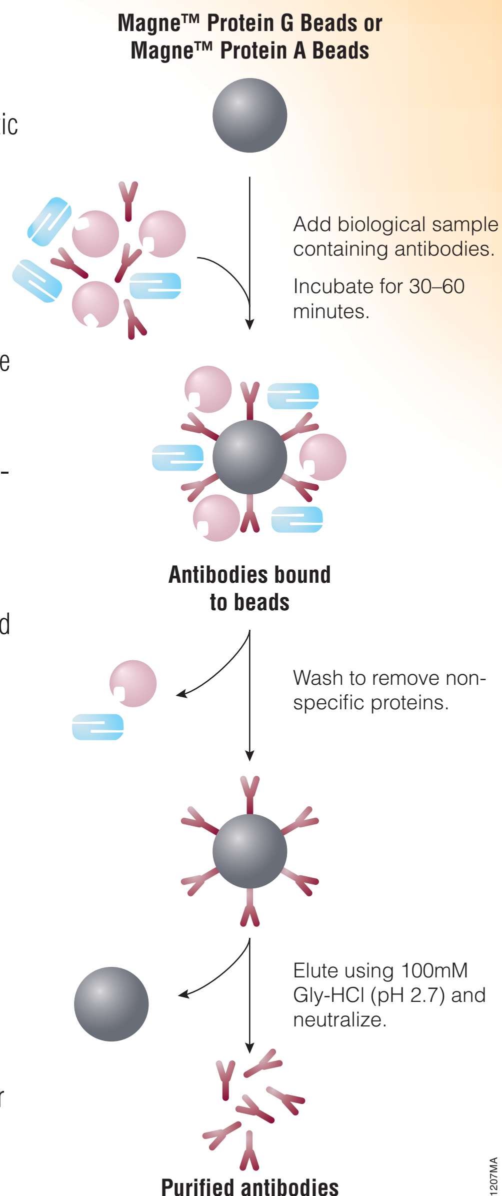
   Note: Magne™ Protein A Beads and Magne™ Protein G Beads are compatible with several bind/was buffers including 25mM sodium acetate (pH 6.0), PBS (pH 7.4) and Tris-buffered saline (pH 7.5)
5. Combine 50µl of bind/wash buffer and 50µl of sample, then add to the equilibrated beads.
6. Mix sample for 30-60 minutes at room temperature. Make sure the beads remain in suspension by using a tube shaker or end-over-end mixer.
7. Place tube in the magnetic stand for 10 seconds. Remove the supernatant and save for analysis if desired.

Wash

8. Add 500µl bind/wash buffer and mix for 5 minutes. Place in the magnetic stand for 10 seconds. Remove and discard bind/wash buffer.
9. Repeat Step 8 for a total of two washes.
10. Add 200µl bind/wash buffer. Mix and place in the magnetic stand for 10 seconds. Remove and discard all bind/wash buffer.

Elute

11. Add 50µl elution buffer [100mM glycine-HCl (pH 2.7)] to the beads.
12. Mix for 5 minutes at room temperature.
13. Place tube in the magnetic stand for 10 seconds. Remove eluted sample and transfer to a new microcentrifuge tube containing 10µl of neutralization buffer [2M Tris buffer (pH 7.5)]. This is the first elution.
14. Repeat elution Steps 11-13. Eluted samples can be combined.
15. Quantitate the amount of antibody recovered using absorbance at 280nm or ELISA. Check the purity of the antibody using SDS polyacrylamide gel electrophoresis.



Additional information is available in Technical Manual #TM371, available online at ***www.promega.com/protocols***

ORDERING/TECHNICAL INFORMATION:
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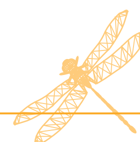

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Quick PROTOCOL

Antibody Purification Guidelines for Various Sample Sizes

Sample Type	Serum, Ascites, Cell Culture Media	Cell Culture Media	Cell Culture Media	Cell Culture Media	Serum, Ascites, Cell Culture Media
Format	1.5ml tubes	1.5ml tubes	15ml conical	50ml conical	96-well plate
Sample Size	50µl	900µl	9ml	45ml	50µl
Bead Slurry	50µl	50µl	500µl	2.5ml	50µl
Amount of Bind/Wash Buffer to Equilibrate Beads	500µl	500µl	5ml	25ml	3 × 150µl
Magnetic Stand	MagneSphere® Technology Magnetic Separation Stand (Z5331, Z5332, Z5333, Z5341, Z5342, Z5343)		PolyATtract® System 1000 Magnetic Separation Stand (Z5410)		Deep-Well MagnaBot® 96 Magnetic Separation Device (V3031)
Amount of Bind/Wash Buffer to Dilute Sample	50µl of 1X bind/wash buffer	100µl of 10X bind wash buffer	1ml of 10X bind wash buffer	5ml of 10X bind wash buffer	50µl of 1X bind/wash buffer
Bind Time	30-60min at room temperature with constant mixing. The beads need to be in suspension for maximal capture. Longer bind times may be necessary at 4°C.				
Wash with Bind/Wash Buffer	2 x 500µl then 1 x 200µl		2 x 5ml, then 1 x 1ml	2 x 25ml then 1 x 5ml	3 x 150µl
Elution Buffer	2 x 50µl		2 x 500µl	2 x 2.5ml	2 x 50µl
Neutralization Buffer	20µl (10µl for each 50µl elution buffer)		200µl (100µl for each 500µl elution buffer)	1ml (500µl for each 2.5ml elution buffer)	20µl (10µl for each 50µl elution buffer

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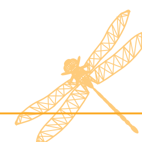

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