Version: 03
Update (MM/DD/YYYY): 12/02/2024

AmMag™ Protein A Magnetic Beads
Cat No: L00695

Index

I Product Description

1. Intended Use

GenScript AmMag™ Protein A Magnetic Beads are developed and optimized for antibody purification for high throughput and high volume applications.

2. Principle

The sample containing antibody is added to the AmMagTM Protein A Magnetic Beads and incubated on a shaker overnight or 2 hours (depending on the concentration of samples) for the antibody to bind to the beads. The antibody bound to the beads can be eluted off by using acidic elution facilitated by a magnetic separation device. Magnetic separation eliminates the need for centrifugation, minimizes the loss of sample and makes the process more user friendly. The AmMagTM protein A Magnetic Beads have a binding capacity of 40 mg human IgG per 1 mL settled beads. They can be reused for up to 40 cycles.

Protein A, a bacterial cell wall protein isolated from *Staphylococcus aureus*, binds to mammalian IgG's, mainly through the Fc regions. The alkaline tolerant protein A not only keeps the specific binding capacity to Fc region of immunoglobulin molecules, but also tolerates alkaline conditions and can withstand rigorous cleaning procedures (See section II.4 CIP for details).

3. Description of Material

➢ Material Supplied

GenScript AmMag™ Protein A Magnetic Beads are super paramagnetic beads with diameter of 45-100 μm, covalently coated with alkaline tolerant protein A. The beads are supplied as 25% slurry in 20% ethanol.

➢ Additional Material Required

Rotor/Rocker (if the proteins are already secreted) or orbital shaker (if the beads are added when the proteins are secreting)
Magnetic Separation Rack (L00722 for AmMag™MR-mini and L00723 for AmMag™MR)
Test tubes and pipettes
Buffers and solutions (see below)

➢ Additional Buffers Required

Binding/Wash Buffer: 1×PBS, 0.1% Tween 20 (optional), pH 7.0
Elution Buffer: 1. 0.1 M glycine, pH 2-3
2. 0.1 M NaAc-HAc, pH3.6
Note: Please use one of the above mentioned buffers for elution.

GenScript USA, Inc.
860 Centennial Ave. Piscataway, NJ 08854
Tel: 1-732-885-9188

Neutralization Buffer: 1 M Tris, pH 8.5
Regeneration Buffer: 0.1M NaOH

II Protocol

The protocol uses 100 µl AmMag™ Protein A Magnetic Beads for purification of 1 ml human IgG sample. Depending upon your sample volume and antibody concentration, additional optimization may be required to determine the volume of magnetic beads optimal for purification.

Working capacity(mg/ml)				
Sample concentration(hIgG)	RT/2hour	37ºC/overnight	4 ºC/2hour	4 ºC/overnight
0.01mg/ml	2.5	2.5	2.5	2.5
0.02mg/ml	5	5	5	5
0.1mg/ml	20	30	15	20
0.5mg/ml	35	60	30	50
1mg/ml	45	70	40	60
3mg/ml	70	100	60	85

1. Preparation of the MagBeads

1) Completely resuspend the beads by shaking the vial.
2) Transfer 400ul slurry (100 µl settled beads in 400µl slurry) into a clean tube.
3) Place the tube on a magnetic separation rack to collect the beads. Remove and discard the supernatant.
4) Add 1 ml Binding/Wash Buffer to the tube and invert the tube several times to mix. Use the magnetic separation rack to collect the beads and discard the supernatant. Repeat this step twice.
(For customers need endotoxin control, please proceed with the following steps.)
5) Add 1 ml 0.1M NaOH to the tube with magnetic beads and incubate for 15min. Then use the magnetic separation rack to collect the beads and discard the supernatant, repeat this step twice.
6) Add 1 ml Binding/Wash Buffer to the tube and invert the tube several times to mix. Use the magnetic separation rack to collect the beads and discard the supernatant. Repeat this step twice.

2. Separation of target IgG

1) Add the sample containing target IgG into the tube and gently invert the tube to mix.
2) Incubate the tube at room temperature with mixing (on a rocker or rotator) for 1~4hours or overnight.
Note: If the protein of interest is unstable and prone to degradation 100mM of PMSF may be added to prevent protein degradation.
3) Use the magnetic separation rack to collect the beads and discard the supernatant. If necessary, keep the supernatant for analysis.
4) Add 1 ml Binding/Wash Buffer to the tube and mix well, use the magnetic separation rack to collect the beads and discard the supernatant. Repeat the wash step three more times.

3. Elution of Isolated IgG

1) Add 500 µl Elution Buffer to the tube and mix well. Incubate for five minutes at room temperature with occasional mixing.
2) Use the magnetic separation rack to collect the beads and transfer the supernatant that contains the eluted IgG into a clean tube.
3) Repeat Step 1 and 2 twice.
4) Add Neutralization Buffer to each 500 µl eluate to neutralize the pH. If needed, perform a buffer exchange by dialysis or desalting.

4. CIP

CIP (clean in place) method is performed after each cycle.The magnetic beads were first cleaned with 500 µL 1 X PBS, Place the tube on a magnetic separation rack to collect the beads. Remove and discard the supernatant.Then incubated with 1 mL 0.1 M NaOH for 15 minutes. After the magnetic beads are adsorbed, discard the supernatant and add another 1 mL of 0.1 M NaOH for further treatment for 15 min. Place the tube on a magnetic separation rack to collect the beads. Remove and discard the supernatant. Then rinse the magnetic beads with PBS and store in 20% ethanol.

GenScript USA, Inc.
860 Centennial Ave. Piscataway, NJ 08854
Tel: 1-732-885-9188

III Applications

1. Application 1: Alkali Resistance Test of AmMag™ Protein A Magnetic Beads

Regenerate AmMag™ Protein A Magnetic Beads with 0.1M NaOH and test their capacity using conventional regeneration procedures.

experiment process:

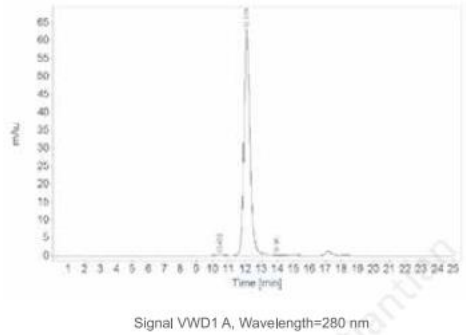
1. Add 5 MV PBS to wash the magnetic beads 3 times (Note: MV refers to the volume of settled magbeads added);
2. Add 20 MV 0.1M NaOH to soak the magnetic beads for 15 minutes, repeat twice for a total of 30 minutes;
3. Add 5 mv PBS to wash the magnetic beads 3 times.

Saturated Capacity after Alkaline-treatment Cycles					
Sample	hIgG:5 mg/mL, 5 mL	hIgG:5 mg/mL, 5 mL	hIgG:5 mg/mL, 5 mL	hIgG:5 mg/mL, 5 mL	hIgG:5 mg/mL, 5 mL
AmMag™ Protein A Magnetic Beads Volume	0.1 mL	0.1 mL	0.1 mL	0.1 mL	0.1 mL
Incubation time	1 h	1 h	1 h	1 h	1 h
Cycles of NaOH treatment	0	10	20	30	40
Binding capacity(mg/mL)	91.71	76.57	74.82	73.17	73.48
Precentage drop in load		15.14%	18.42%	20.34%	19.88%

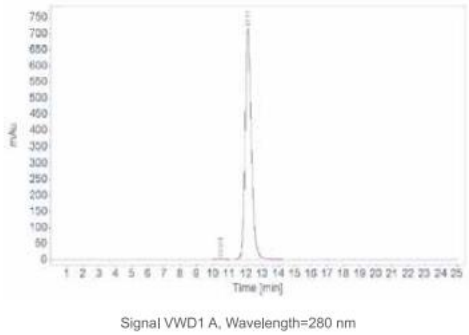
Conclusions: After AmMag™ Protein A Magnetic Beads alkali treatment was repeated 40 times, the percentage drop in binding capacity loss was less than 20%.

2. Application 2: Comparison of hIgG1 purification results

Purification method	Beads Volume	Protein Name	Host cell line	Concentration of the samples	Samples Volume	Elution volume	Elution cycle	Recovery	Purity(SEC-HPLC)
AmMag™ Protein A Magnetic Beads Volume	0.5 mL	IgG1λ	HEK2 93-6E	153.83 mg/L	50 mL	1.1 mL	2	7.729 mg	99.36%
Resin-by Tecan	0.6 mL	IgG1λ	HEK2 93-6E	153.83 mg/L	50 mL	0.7 mL	5	7.818 mg	99.09%



AmMag™ Protein A Magnetic Beads



Resin by Tecan

Conclusions: AmMag™ Protein A Magnetic Beads can obtain the same purity and recovery comparable to resin in purifying hIgG1 samples.

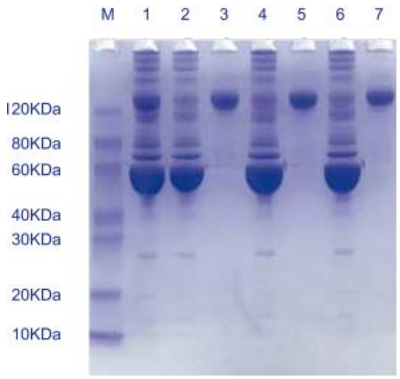
GenScript USA, Inc.
860 Centennial Ave. Piscataway, NJ 08854
Tel: 1-732-885-9188

3. Application 3: Antibody Purification from Rabbit Serum

Sample(M087-1, Mouse IgG2b, k)	Dilute 10 mL Mouse ascites with 10 mL PBS
MagBeads	AmMag™ Protein A Magnetic Beads
MagBeads volumn	1 mL
Wash Buffer	PBS
Elution Buffer	0.1 M Acetic Acid-Sodium Acetate,pH 3.6
AmMag™ SA	Cat No.L00768

Sample(M087-1, Mouse IgG2b, k)	Dilute 10 ml Mouse ascites with 10ml PBS
Resin	1ml Monofinity A Resin Prepacked column
Wash Buffer	PBS
Elution Buffer	0.1M Acetic Acid-Sodium Acetate,pH3.6
AKTA	Pure-25

Instrument	Sample Processing Method	1st Elution volumn (mL)	1st Elution concentration (mg/mL)	2nd Elution volumn (mL)	2nd Elution concentration (mg/mL)	3rd Elution volumn (mL)	3rd Elution concentration (mg/mL)	Antibody recovery (mg)
AmMag™SA	uncentrifugal and unfiltration	3.6	2.423	3.66	0.586	3.66	0.227	11.698
AmMag™SA	centrifugal and filtration	3.79	2.439	3.62	0.427	3.7	0.104	11.174
AKTA	centrifugal and filtration	4.4	2.533	/	/	/	/	11.145



Lane M: Marker(M00516)
Lane 1: Sample 1 ul
Lane 2: Resin-Flow through 1 ul
Lane 3: Resin-Elution 5 ug
Lane 4: Magnetic Beads-uncentrifugation and unfiltration
-Supernatant after incubation 1 ul
Lane 5: Magnetic Beads-uncentrifugation and unfiltration
-1 st to 3 rd Elution mixture 5 ug
Lane 6: Magnetic Beads- centrifugation and filtration
-Supernatant after incubation 1 ul
Lane 7: Magnetic Beads- centrifugation and filtration
-1 st to 3 rd Elution mixture 5 ug

Conclusions: AmMag™ Protein A Magnetic Beads can obtain the same purity and recovery comparable to resin.

IV Troubleshooting

Review the information below to troubleshoot your experiments using the GenScript AmMag™ Protein A Magnetic Beads .

Problem	Possible Cause	Solution
The beads are difficult to immobilize using the magnetic separation rack.	Too many beads are used.	Decrease the volume of magnetic beads used.
A considerable amount of sample has been added, but very little specific antibody of interest is detected.	The antibody of interest is at a very low concentration.	Use a serum-free medium for cell supernatant samples. Bind the samples to the beads overnight at 4°C. Affinity-purify the antibody using its specific antigen coupled to an affinity supporting material.

GenScript USA, Inc.
860 Centennial Ave. Piscataway, NJ 08854
Tel: 1-732-885-9188

The antibody of interest is purified, but it is degraded (as determined by loss of function in downstream assay).	The antibody is sensitive to low-pH elution buffer. The downstream application is sensitive to the neutralized elution buffer.	Try another elution reagent, such as 0.1M Sodium acetate, pH3.6 Add protease inhibitor to the binding, washing and elution buffers. Desalt or dialyze the eluted sample into a suitable buffer.
No antibody is detected in any eluate.	The antibody in the sample cannot bind to Protein A.	Try GenScript Protein G MagBeads or Protein A/G MagBeads.

V General Information

1. Storage and Stability

This product is stable until the expiration date of 2 years, when stored unopened at 2-8°C. **Do NOT freeze the product**. Keep the magnetic beads in liquid suspension during storage and all handling steps. Drying will cause loss of binding capacity and result in reduced performance. Resuspend the beads well before use.

2. Technical Support

Please contact GenScript for further technical information (see contact details). Certificate of Analysis/Compliance is available upon request. The latest revision of the package insert/instructions for use is available on www.genscript.com.

3. Warning and Limitations

This product is for research use only. Not intended for any animal or human therapeutic or diagnostic use unless otherwise stated. This product contains 20 % EtOH as a preservative. Flammable liquid and vapor. Flash point 38°C. R-10 flammable. Material Safety Data Sheet (MSDS) is available at http://www.genscript.com.

4. Related MagBeads Products

Cat. No.	Product Name
L00672	Protein A MagBeads MX
L00673	Protein G MagBeads MX
L00274	Protein G MagBeads
L00277	Protein A/G MagBeads
L00295	Ni-Charged MagBeads
L00776	AmMag™ Ni Magnetic beads
L00936	Streptavidin MagBeads
L00895	Glutathione MagBeads
L00722	AmMag™ MR-mini Magnetic Rack
L00723	AmMag™ MR Magnetic Rack

For research use only. Not intended for human and animal therapeutic or diagnostic use.