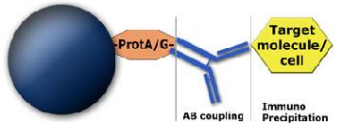
MagSi-protein A 600, 1.0, 3.0 Art.No.: MDXX011



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Product Description

I. Intended Use

MagSi-protein A is intended for capture of immunoglobulins, for purification purposes or for downstream use including antibody screening, immunoprecipitation, protein interaction studies, phage display, immunoassays, and cell isolation. MagSi-protein A is produced using a recombinant protein A fusion protein (~45 kDa), derived from E. coli fermentation, resulting in minimum non-specific binding.

Protein A binds to the Fc region of immunoglobulins, but has different binding strength for specific species and Ig subclasses (Table 1). Immunoglobulins optimally bind to Protein A at near neutral pH (~8), and can be released at low pH (2-3) or in protein denaturing conditions.

II. Principle

MagSi-protein A beads are added to a sample containing immunoglobulins. During a short incubation, the immunoglobulins will bind to MagSi-protein A via their Fc part. Afterwards the complex is separated from the sample using a magnet and the beads are washed to remove unbound molecules. Purified immunoglobulins can be eluted or the complex can be used in downstream applications.

III. Material Supplied

Vial with 1 or 5 mL of the following products:

- MagSi-Protein A 600 MD10011: 1 mL, MD11011: 5 mL
- MagSi-Protein A 1.0 MD01011: 1 mL, MD02011: 5 mL
- MagSi-Protein A 3.0 MD41011: 1 mL, MD42011: 5 mL

All products are supplied at 10 mg/mL in PBS, 0.05% sodium azide.

IV. Product Use

When stored at 2-8°C, this product is stable up to 2 years, but no longer than the expiry date on the label. Store in well closed vial and in upright position to prevent drying of the beads, this may result in a decrease of activity. Do not freeze the product! Vortex well before use. Wash the beads to remove preservatives that could interfere with your application.

Table 1: Binding strength to Protein A

Species	Antibody Class	Binding to Protein A
Human	Total IgG	++++
	IgG1	+
	IgG2	++++
	IgG3	++++
	IgG4	++++
Mouse	Total IgG	++++
	IgG1	+
	IgG2A	++++
	IgG2B	++++
	IgG3	+++
Rat	Total IgG	+
	IgG1	-
	IgG2A	-
	IgG2B	-
	IgG2C	++
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	-

Additional materials needed:

- Consumables (depending on the application, contact for support)
- Magnetic separator (see order information)
- Mixer/vortex shaker to homogenize samples and resuspend beads (depending on the application, contact for support)

Washing, Binding and Elution Buffers:

- Washing and Binding Buffer: For binding of antibodies a neutral buffer (PBS) is recommended, optionally with a surfactant (Tween20/Triton X-100/NP40) and 0.1% BSA to reduce background absorption.
- Ig Elution Buffer: 0.1 M glycine or 0.1 M citrate (pH 2-3)
- Neutralization buffer: 1 M Tris pH 9

V. Protocols

A) Preparation of beads

Optimize the quantity of beads for each application

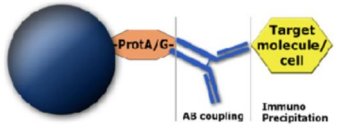
1. Resuspend beads by shaking/vortexing
2. Add 100 µL of MagSi-protein A beads into a microtube.
3. Add 300 µL of Binding/Washing Buffer to the tube and gently mix. Collect the beads and then discard the supernatant. Repeat this step twice. Finally resuspend in 90 µL Binding Buffer.

B) Ig Capture

Maximum binding of Ig is usually obtained after 45 minutes. However, for some applications 15 minutes might be sufficient.

Binding of Ig to protein A is an equilibrium reaction. It is important to maintain high concentrations of beads and Ig.

In case the volume of serum sample is more than ¼ of the



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bead volume, equilibrate the sample with 0.5 M Phosphate buffer pH 8 5X stock solution (final concentration = 0.1 M) before adding to the beads (e.g. add 10 µL stock solution to 40 µL serum).

1. Add 10 µL serum sample to the 90 µL beads.
2. Incubate 30 minutes at room temperature with mild agitation.
3. Collect beads by placing the tube on the magnet for 2 minutes, pipette off the supernatant.
4. Remove the tube from the magnet, add 100 µL of Binding/Washing Buffer and resuspend.
5. Repeat step 3 and 4 twice, then place the tube on the magnet for 2 minutes and pipette off the supernatant.

C) Ig Elution

The pH needed for elution depends on the species and Ig subclass, but 0.1 M glycine pH 2.8 (low pH elution) will elute most Ig.

1. Add a suitable volume of 0.1 M glycine pH 2.8 to the tube, e.g. 50 µL.
2. Incubate the tube at room temperature with mixing for 3 minutes.
3. Collect beads on the magnet for 2 minutes and transfer the supernatant containing purified antibodies.
4. Immediately after elution, adjust to physiological pH by adding alkaline buffer (e.g. 1 M Tris pH 9).

D) Immunoprecipitation

If co-elution of the antibody with target antigen is unwanted in the application, the antibody should be crosslinked. Protocols for crosslinking are available by request.

1. Add sample containing antigen to the magnetic beads – Ig complex. Incubate 1-2 hours or overnight at 4°C with mild agitation. If dilution is necessary, use PBS (optionally with a surfactant).
2. Collect beads by placing the tube on the magnet for 1-2 minutes, pipette off the supernatant.
3. Add 300 µL of Binding/Washing Buffer to the tube and resuspend the beads.
4. Collect the beads and then discard the supernatant.
5. Repeat step 3 and 4 twice.

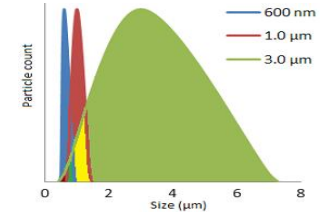
E) Elution of antigen

Different elution methods can be used to release antigens from the magnetic beads – Ig complex. Low pH (2-3), change in ionic strength, denaturing buffers (SDS-PAGE) and other methods can be applied. The method depends on the Ig's affinity to the antigen, protein stability, and downstream application. Most antigens will be eluted with low pH as described under C).

VI. Technical Data

MagSi-protein A magnetic beads are available in three sizes: 600 nm, 1 µm and 3 µm. The sedimentation time of 600 nm MagSi beads is approximately 4 times longer than that of 1.0 µm beads. MagSi beads with a diameter of 3 µm have stronger magnetic properties and will separate approximately 4x faster than 600 nm beads under the same conditions.

Table 2: Specifications of MagSi-protein A

Product Name	MagSi-protein A 600 / 1.0 / 3.0		
Size	600 nm	1.0 µm	3.0 µm
Concentration	10 mg/mL		
Beads / mL	8 - 20 · 109	6 - 12 · 109	1 - 3 · 109
Material	Magnetic silica beads with Protein A covalently bound to the surface		
Magnetic content	~40%	~60%	~60%
Size distribution	500 - 900 nm	0.7 - 1.4 µm	0.6 - 10.0 µm
(D5-D95)			

VII. Additional Information

Disclaimer

For Research Use Only (RUO). Not for drug, household or other uses. Safety Data Sheet (SDS) is available at www.magtivio.com.

Ordering information

Product	Volume	Art.No.
MagSi-protein A 600	1 mL	MD10011
MagSi-protein A 600	5 mL	MD11011
MagSi-protein A 1.0	1 mL	MD01011
MagSi-protein A 1.0	5 mL	MD02011
MagSi-protein A 3.0	1 mL	MD41011
MagSi-protein A 3.0	5 mL	MD42011

Related products

Product	Art.No.
MM-Separator M12 + 12	MD90001
MM-Separator M96	MD90002

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