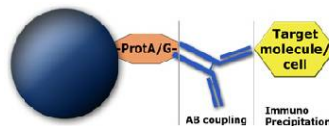


MagSi-protein A and G 600, 1.0 Product Description



I. Intended Use

MagSi-protein G and MagSi-protein A are useful for many applications, including immunoprecipitation, antibody screening, protein interaction studies, phage display, immunoassays, purification of proteins and peptides or nucleic acids, and cell isolation.

Protein A and Protein G bind to the Fc region of immunoglobulins, but have different specificities (Table 1). The albumin, cell wall and cell membrane binding domains, present on the wild-type protein G, have been removed from the recombinant protein G (~26kDa) which is used for MagSi-protein G beads. The 42kDa Staphylococcal Protein A on MagSi-protein A is produced in *E. Coli*. Immunoglobulins optimally bind to Protein A and Protein G at pH 8, and can be released at low pH (2.5-3) or in protein denaturing conditions.

MagSi-protein A and MagSi-protein G beads are offered in sizes of 600 nm and 1.0 μ m. The sedimentation time of 600nm MagSi beads has been optimized and is approx. 4 times longer compared to 1.0 μ m beads. This allows e.g. long incubation times without shaking/mixing etc. MagSi beads with a diameter of 1 μ m have stronger magnetic properties and will separate approx. 2x faster compared to 600nm in the same conditions; the typical separation time is \leq 1 minute using a suitable magnet.

II. Principle

MagSi-protein A or MagSi-protein G beads are added to a sample containing immunoglobulins. The immunoglobulin molecules will bind to the beads during a short incubation after which the complex is separated from the sample using a magnet. 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 MagSi-protein A 600, MagSi-protein A 1.0, MagSi-protein G 600, or MagSi-protein G 1.0 (supplied at 10 mg/ml in PBS, 0.05% Sodium Azide)

Table 1: Immunoglobulin specificities for Protein A and Protein G

Species	Antibody Class	Protein A	Protein G
Human	Total IgG	++++	++++
	IgG ₁	+	++++
	IgG ₂	++++	++++
	IgG ₃	++++	++++
	IgG ₄	++++	++++
Mouse	Total IgG	++++	++++
	IgG ₁	+	+++
	IgG _{2a}	++++	++++
	IgG _{2b}	++++	++++
	IgG ₃	+++	+++
Rat	Total IgG	+	++
	IgG ₁	-	+
	IgG _{2a}	-	++++
	IgG _{2b}	-	++
Hamster	Total IgG	++	++
	IgG ₁	-	-
	IgG _{2a}	-	-
	IgG _{2c}	++	+++
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:

- Buffers and Materials (depending on the application, contact for support)
- Magnetic separator for bead separation/collecting (see order information)
- Mixer/vortex to homogenize samples and resuspend beads (depending on the application, contact for support)

Washing and Binding Buffers :

- For coupling 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.

- For release of antigens from antibodies, glycine 0.1M pH 2.8 (low pH elution) is recommended. Heating above 70°C in reducing SDS-PAGE buffer also releases antigens, but antibody and protein A and G as well.

IV. Product Usage

This product is stable for at least 1 year after purchasing date when stored at 2-8°C. 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.

V. Protocols

A) Bead preparation procedure

1. Resuspend beads by shaking/vortexing
2. Pipette the required volume of beads into a tube or microplate (10-20 μ l is suitable as a starting point)
3. Collect beads by placing the tube or microplate on the magnet for 1-2 minutes
4. While tube/micro plate is still on the magnet, carefully remove supernatant without touching the bead pellet
5. Take tube/micro plate from the magnet and add Washing Buffer
6. Resuspend beads by vortexing or pipetting
7. Repeat step 3 – 5 at least 3 times
8. Finally resuspend the beads in a suitable buffer for your downstream use, in a volume equal to the original volume.

B) Immunoprecipitation

1. Resuspend beads by shaking/vortexing
2. Add 25-50 µl of MagSi-protein A or MagSi-protein G beads into a 1.5 ml microcentrifuge tube.
3. Prepare the beads for binding by washing with Binding Buffer as described in a). Finally resuspend in 500 µl Binding Buffer.
4. Combine the antigen sample with 2-4 µg of IgG. Dilute each sample to a minimum volume of 300 µl with cell lysis buffer or Binding/Wash Buffer. Incubate 1-2 hours at room temperature or overnight at 4°C with mixing.
5. Add the sample mixture to the 1.5 ml microcentrifuge tube containing pre-washed magnetic beads (3.) and incubate at RT for 30 minutes with mixing.
6. Collect beads by placing the tube on the magnet for 1-2 minutes, pipette off and save the supernatant for analysis.
7. 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.
8. Add 50 µl of Elution Buffer to the tube. For low pH elution, incubate the tube at room temperature with mixing for 5 minutes. For SDS-PAGE elution, add 50 µl of SDS-PAGE reducing sample buffer to the tube and incubate the samples at 90°C for 10 minutes.
9. Collect beads by placing the tube on the magnet for 1-2 minutes and transfer the supernatant containing target antigen.

*Low pH elution buffers are effective for most antibody-antigen interactions; however, to ensure efficient release of target antigen, pre-rinse the beads with 300 µl 0.1% Tween-20 in water without buffer before adding low pH elution buffer.
** If co-elution of the target antigen and the antibody is unfavourable in the application the antibody should be crosslinked. Protocols for crosslinking are available from MagnaMedics under request.

C) Antibody purification

1. Resuspend beads by shaking/vortexing
2. Add 100 µl of MagSi-protein A or MagSi-protein G beads into a 1.5 ml microcentrifuge tube.
3. Prepare the beads for binding by washing with Binding Buffer as described in a). Finally resuspend in 90 µl binding buffer.
4. Add 10 µl serum sample. Incubate 1-2 hours at room

temperature or overnight at 4°C with mixing.

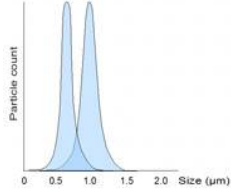
5. Collect beads by placing the tube on the magnet for 1-2 minutes, pipette off and save the supernatant for analysis.
6. Add 100 µl of Binding/Washing Buffer to the tube and gently mix. Collect the beads and then discard the supernatant. Repeat this step twice.
7. Add 50 µl of Low pH elution buffer to the tube. Incubate the tube at room temperature with mixing for 5 minutes.
8. Collect beads on the magnet for 1-2 minutes and transfer the supernatant containing purified antibodies.

Practical Notes:

- Optimize the quantity of beads for each application
- In case the volume of sample for C) is more than ¼ of the bead volume, equilibrate the sample first with a 0.5 M Phosphate buffer pH 8 5X stock solution (final molarity 0.1 M).

VI. Technical Data

Table 2: Specifications of MagSi-protein A and MagSi-protein G

Product Name	MagSi-Protein A 600 MagSi-Protein G 600	MagSi-Protein A 1.0 MagSi-Protein G 1.0
Size	600 nm	1.0 µm
Concentration	10 mg/ml	
	beads/ml	
	8 - 20 · 10 ⁹	6 - 12 · 10 ⁹
Material	Magnetic silica beads with Protein A or Protein G covalently bound to the surface	
Size Distribution	D5-D95	
	500 - 900 nm	0.7 - 1.4 µm
		
Solution additives	PBS (pH 7.4), 0.05% Sodium Azide (NaN ₃ , Toxic!)	
Storage	Store at 2-8°C	

VII. Additional Information

Disclaimer

For R&D use only. Not for drug, household or other uses. Product contains 0.05% Sodium Azide which is toxic. Avoid contact with the suspension buffer. When disposing the suspension buffer, flush with large amounts of water. Material Data Sheet (MSDS) is available on our website at www.magnamedics.com.

Order 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 G 600	1 ml	MD10012
MagSi-protein G 600	5 ml	MD11012
MagSi-protein G 1.0	1 ml	MD01012
MagSi-protein G 1.0	5 ml	MD02012

Related products

Product	Art.No.
MM-Separator M12 + 12	MD90001
MM-Separator M96	MD90002
MM-Separator 96 SBS	MD90005
MM-Separator 384 SBS	MD90006

MagnaMedics Diagnostics B.V.

Burg, Lemmensstraat 366
6163 JT Geleen
The Netherlands
www.magnamedics.com
E-mail: info@magnamedics.com
tel: +31(0)46 820 0206
fax: +31(0)46 410 6825