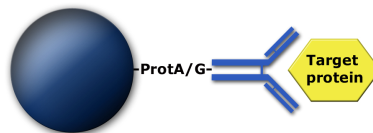
MagSi-protein A and G 600, 1.0, 2
Product Description



Product nr: MDXXX11, MDXXX12, MDXXX13

Technical Data

Product Name	**MagSi-Protein A/G**		
	600	1.0	2
Size	600 nm	1.0 µm	2 µm
Concentration	10 mg/ml		
	beads/ml		
	7-12 · 10^9	3 -8 · 10^9	0.8 -2 · 10^9
Supplied volume	1 ml, 5 ml		
Material	Magnetic silica beads with Protein A or G covalently bound to the surface		
Size Distribution	CI 90%		
	500 – 900 nm	0.7 – 1.3 µm	0.8 – 3.5 µm
Magnetic Particle Content (per gram dry solid)	30-40%		
Solution additives	PBS (pH 7.4), 0.05% Tween20, 0.05% Sodium Azide (NaN_3, Toxic!)		
Storage	Store at 4-8°C		

Material Supplied

- Vial with Protein A and/or Protein G coated MagSi beads suspended in PBS buffer containing 0.05% Tween20 and 0.05% Sodium Azide. Magsi-Protein A/G is a 50% mix of protein A and protein G coated beads

Application

General Information

MagSi-protein G and MagSi-protein A beads are magnetic silica beads coated with protein G or protein A.

The recombinant protein G (~26kDa) on the surface of our beads contain only IgG binding sites. The albumin, cell wall and cell membrane binding domains, present on the wild-type protein G, have been removed to optimize binding properties. The 42kDa protein A is from *Staphylococcus aureus.*

Protein A/G coated beads can be used to detect or isolate target proteins by coupling the Fc domain of the IgG antibody for a target protein. The advantage of protein G an A beads, compared to Streptavidin beads, is that the antibody does not need to be biotinylated and the binding is reversible. So they are ideal for protein isolation and proteomics. Also, the magnetic properties allow easy and quick washing steps in protein isolations.

Binding affinity of Protein A and Protein G

IgG	**Protein A**	**Protein G**
Human	Strong	Strong
Mouse	Strong, IgG1 weak	Strong
Rat	Weak	Strong
Goat	Weak/No binding	Strong
Ginea Pig	Strong	Weak
Rabbit	Strong	Strong
Bovine	Weak	Strong
Sheep	Weak	Strong
Horse	Weak	Strong
Dog	Strong	Weak
Cat	Strong	No binding
Pig	Strong	Strong

Bead Usage

This product is stable for at least 1 year after production date when stored at 2-8°C. Store beads in well closed vial and in upright position to prevent drying of the beads since this makes them more difficult to resuspend and may decrease their activity. Do not freeze the product! Vortex bead suspension well before use. If you expect iron interference in downstream applications, we strongly advise you to rinse the beads before usage.

The MagSi-protein A and G beads are suspended in PBS buffer + 0.05% Tween and contains 0.05% sodium azide as a preservative. Before using the beads it's important to rinse them with PBS/Tween solution to remove the NaN_3 that could interfere with your test and for safety reasons since NaN_3 is toxic! MSDS of our products can be found at our site (www.magnamedics.com)

We recommend to use PBS with ± 0.05% Tween (or another surfactant) for washing when using them in a matrix with proteins. The presence of Tween reduces the protein background absorption to an absolute minimum.

1. Resuspend beads by shaking/vortexing
2. Pipette needed amount in tube or micro plate
3. Collect beads by placing the tube or micro plate on the magnet for 1 - 2 minutes
4. While tube/micro plate is still on the magnet, carefully remove supernatant without touching the pellet of beads
5. Take tube/micro plate from the magnet and add appropriate amount of washing buffer (+/- 50 µl for a micro plate and +/- 200 µl when using a 1.5 - 2 ml tube).
6. Resuspend beads by vortexing or pipetting
7. Repeat step 3 – 5 at least 3 times.

Additional materials needed

Depending on the application, some buffers and materials are needed.

- Magnets for bead separation/collecting.
- Buffers. As wash and binding buffer we recommend a neutral buffer (PBS) with a surfactant (0.05% Tween20) to reduce background absorption.
- Mixer/vortex to mix samples and resuspend beads

Protocols

Target capture

Method:

1. Use fresh or frozen cell pellet of 10^6-10^7 cells
2. Lyse cells with 5ml lysis buffer until it's a clear solution (Lysis buffer: 50mM Tris-HCl (pH7.5), 200 mM NaCl, 5mM EDTA, 0,1% Triton X100); store on ice for step 9.
3. Pipette 20µl beads in a 2ml centrifuge tube
4. Wash at least 2 times with PBS/Tween buffer and resuspend in 100µl PBS/Tween
5. Bind IgG to beads by adding 1µg of IgG
6. As control for background absorption, also incubate beads with PBS only, so don't bind IgG to these beads
7. Incubate 1 hour at room temperature
8. Wash beads at least 3X with PBS/Tween
9. Add 250µl of cell lysate, incubate 1 hour at room temperature
10. Wash at least 4X with PBS/Tween, remove supernatant

Total elution by SDS

As an example, GAPDH (glyceraldehyde-3-phosphate Dehydrogenase) was chosen. GAPDH is one of the so called housekeeping genes and is always expressed in a cell. This protein can be used as a reference when studying the level of protein expression in a cell.

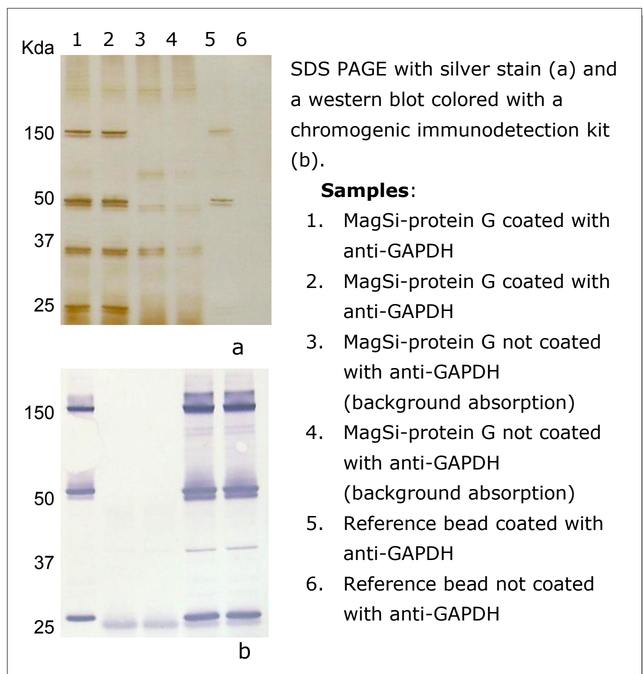
Method:

1. Add 20µl of 1X SDS buffer with reducing agent (reducing agent like DTT will break up the subunits of proteins)
2. Incubate 10 minutes at 60-70°C
3. Collect beads and transfer supernatant to new centrifuge tube
4. Perform SDS-PAGE (and western blot) to analyse your protein.

Results

Lane 1 and 2 clearly shows the different subunits of the anti-GAPDH IgG (150 kDa whole molecule, 50 kDa heavy chain and 25 kDa light chain). In-between the heavy and light chain of the IgG, the 37 kDa GAPDH band is visible. The Western blot and chromogenic immunodetection with anti-GAPDH as primary antibody and a POD labelled second antibody confirms this band is GAPDH.

The SDS PAGE gel, as well as the western blot, shows that our MagSi beads perform very wll since the bands of IgG and GAPDH are very bright.



SDS PAGE with silver stain (a) and a western blot colored with a chromogenic immunodetection kit (b).

Samples:

1. MagSi-protein G coated with anti-GAPDH
2. MagSi-protein G coated with anti-GAPDH
3. MagSi-protein G not coated with anti-GAPDH (background absorption)
4. MagSi-protein G not coated with anti-GAPDH (background absorption)
5. Reference bead coated with anti-GAPDH
6. Reference bead not coated with anti-GAPDH

Target elution

The target protein can be eluted from the protein G and A under acidic conditions. Since the MagSi beads are not stable at extreme pH values (≤2), IgG can be eluted at pH=3

1. Add 50µl of 0.1M glycine buffer (pH 2.6) to the beads
2. Mix gently for 5 minutes at room temperature
3. Collect beads with a magnet and transfer aliquot, containing target protein in a new tube
4. Repeat step 1-3 to elute maximum amount of target protein from the beads

Additional Information

Internet

- www.magnamedics.com

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	Product number
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 2	1 ml	MD04011
MagSi-protein A 2	5 ml	MD05011
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
MagSi-protein G 2	1 ml	MD04012
MagSi-protein G 2	5 ml	MD05012

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