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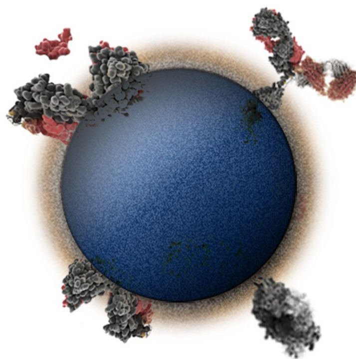
MagSi-Direct 3.0

Art.No.

MD41029 – MD43029 - MD44029



Product Manual

Version 1.0 | 09/08/2018

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MagSi-Direct

Couple the biological molecule of your choice

This product is for Research Use Only (RUO). Not for drug, household or other uses. For more information, please consult the appropriate Safety Data Sheet (SDS), available on our website at www.magtivio.com

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1. General information

1.1 Overview

MagSi-Direct enables coupling of the biological molecule of your choice to magnetic beads. The coating of MagSi-Direct acts as a nanoglue, using electron donation from electron-rich groups of the target molecule including -COOH, -CONH-, -NH2, -NHR-, -NR2, -OH, -SH, -X. As a result, MagSi-Direct allows coupling of a wide variety of medium-large biological molecules, ranging from proteins such as antibodies, cell receptor proteins, lectins, peptide aptamers and enzymes to non-protein molecules. The coating technology has a maximum interaction with large biomolecules.

After the coupling reaction is complete, the functionalized beads may then be used for immunoassays, cell isolation or removal, immunoprecipitation, protein purification and many other applications. Captured proteins, protein complexes, cells, and other targets are easily separated from a solution using the magnetic properties of MagSi beads, enabling washing, buffer exchanges and elution.

With a mean diameter of 3 µm, MagSi-Direct 3.0 is especially suitable for cell capture applications and capture reactions in high volumes or viscous samples.

1.2 Advantages of MagSi-Direct

- MagSi-Direct is a complete kit for custom functionalization including paramagnetic MagSi beads and surface coating solutions
- Low cost and simple coupling procedure
- Highly efficient coupling of most biological molecules to magnetic beads
- Non-covalent coating allows efficient and delicate binding of many sensitive ligands, which is strong even in extreme conditions
- No expensive laboratory tools required, magnetic beads enable easy processing and automation for many applications
- Functionalized beads can be stored in various aqueous buffers.

2. MagSi-Direct contents

Article Number	MD41029	MD43029	MD44029
MagSi-Direct 3.0 Particle Mix	2 mL	10 mL	100 mL
Immobilization Buffer (10X)	4 mL	20 mL	200 mL
Blocking Buffer	1 mL	5 mL	50 mL
Product Manual	1	1	1

2.1 Materials Supplied by the User

Consumables & Equipment	
Micropipettes	200 µL and 1000 µL
Centrifuge tubes	1.5 or 2 mL
Magnetic separator	MM-Separator M12 + 12 (magtivio, Art.No.: MD90001: Magnetic separator for 1.5 and 2 mL centrifuge tubes)
Mixing equipment	Vortexer and rotating mixer for centrifuge tubes

3. Product Usage

3.1 Storage

When stored at 2-8°C the kit is stable for up to 1 year, but no longer than the expiry date on the label. Do not freeze!

3.2 Disclaimer

For Research Use Only. Not for human or animal therapeutic use or diagnostics use.

3.3 Safety information

Wear a suitable lab coat, disposable gloves and protective goggles. For more information, please consult the appropriate safety data sheets (SDS). These are available online at www.magtivio.com.

3.4 Considerations for coupling

Ligand use

MagSi-Direct 3.0 allows optimal coupling at low concentrations of antibody or protein (~5 µg/mg beads). Low affinity ligands may require increasing the amount of input.

The optimal coupling range is ligand dependant and determined empirically. Using excess ligand significantly increases consumption. If it is not a cost factor, it is recommended to use u to 50 µg per mg beads. However, an excess of the ligand will increase the potential for leakage in downstream use.

The presence of antibody aggregates also increases the potential for leakage. This effect can be reduced by removing the aggregates by centrifugation at 16,000 x g for 10 minutes at 4°C.

Different biological molecules such as antibodies, proteins, enzymes, etc. have different characteristics. Even antibodies raised in the same species against the same antigen can vary greatly. As a consequence, the coupling efficiency may vary between different batches. The stability is also dependent on ligand used.

In order to optimize the coupling of antibodies/proteins, the user should consider the buffer composition, preservatives and stabilizing proteins used to store the antibodies/proteins.

Buffer composition

In case the storage buffer of the biological molecules contain amine groups within their ingredients (e.g. Tris), the buffer may need to be exchanged with 1X Immobilization Buffer by dialysis prior to coupling antibodies/proteins to the beads.

Preservatives

Some preservatives included in buffers (sodium azide, thimerosal) may lead to a small decrease in antibody coupling efficiency. For most applications it will not have a significant effect, but if necessary it can be compensated by slightly increasing the quantity of antibody used in the coupling reaction. Alternatively, preservatives can be removed prior to coupling by standard gel filtration chromatography or dialysis against 1X Immobilization Buffer.

Stabilizing reagents

Storage buffers of antibodies, proteins and enzymes may contain protein additives such as BSA or gelatin. If untreated, these additives may be coupled to the bead surface along with the antibody or protein.

Glycerol

Some proteins (enzymes) are supplied in glycerol. Coupling of antibodies/proteins stabilized in glycerol is not recommended. Although it is possible, the functionality may be negatively effected. Glycerol can be exchanged with 1X Immobilization Buffer by dialysis prior to coupling.

3.5 Scale of coupling reaction

The protocol included is easily scalable to the preferred amount of beads for your application. The standard protocol is suitable to prepare 1 mL of functionalized beads (10 mg/mL), but can be scaled up or down by increasing or decreasing all reaction components proportionally.

4. Coupling Protocol

4.1 Preparations before use

- Prepare a 1X Immobilization Buffer:

  Dilute the 10X Immobilization Buffer (0.25 M MES pH 5.5 Proclin 300 0.5%) stock solution 10x in ddH_2O

- The Blocking Buffer included in the kit contains BSA as a blocking agent. If the user requires a different blocking agent (gelatin, HSA, casein, etc.), prepare a Blocking Buffer containing the blocking agent of choice in 1X Immobilization Buffer (the standard Blocking Buffer is the same as 1X Immobilization Buffer, but includes 0.1% BSA)

4.2 Procedure

Part I. Aliquot and wash the beads

1. Vortex MagSi-Direct beads to fully resuspend the beads.
2. Aliquot 1 mL of **MagSi-Direct 3.0 Particle Mix** to a 1.5 mL tube.
3. Place the tube on the magnetic separator for 2 minutes to collect the magnetic beads and discard the supernatant.
4. Wash the beads twice with 1 mL 1X Immobilization Buffer. Vortex, separate and discard the supernatant each time. Resuspend the beads in 250 µL 1X Immobilization Buffer and vortex thoroughly to resuspend the beads.
5. Prepare a solution of the antibody or protein in 1X Immobilization Buffer. Use a final concentration of 400 µg/mL.

   (Example: Mix 125 µL ddH_2O and 25 µL 10X Immobilization Buffer, and add 100 µL protein stock solution of 1000 µg/mL. For optimal coupling conditions of the antibody or protein, see 3.3; Considerations)

6. While continuously mixing the protein solution at moderate speed, slowly add the washed bead suspension from step 4. Incubate for 60 minutes at room temperature on a rotating mixer.
7. Wash twice with 1 mL 1X Immobilization Buffer. Vortex, separate and discard the supernatant each time.
8. Add 1 mL of Blocking Buffer and vortex at moderate speed for 10 seconds. Incubate for 60 minutes at room temperature on a rotating mixer.
9. Collect the magnetic beads using the magnetic separator and discard the supernatant.
10. Wash twice with 1 mL 1X Immobilization Buffer. Vortex, separate and discard the supernatant each time.
11. Add the 1 mL of ddH_2O, 1X Immobilization Buffer or a different storage buffer of choice depending on the application. Optionally add a detergent. In case the beads are stored, add a preservative.
12. Store the beads at 2-8°C until use. Do not freeze.

5. Troubleshooting

Troubleshooting guidelines for MagSi-Direct

Problem	Possible cause	Suggestion
Spillage of beads	Mixing too intensive	- Determine the proper speed with a volume of water equal to the total volume of the coupling reaction. Apply these settings when mixing the ligand solution and adding Magsi-Direct beads
No detectable functionality after coupling of biological molecule	Inefficient coupling of target	- Evaluate the coupling considerations in chapter 3.4 - Make sure to continuously mix the ligand solution while adding MagSi-Direct
Unspecific binding to functionalized beads upon use	Blocking procedure unsuccesful	- Blocking Buffer containing BSA is not suitable for the application. Select a suitable agent for surface blocking and prepare a solution in 1X Immobilization Buffer.

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