MagSi-proteomics C4, C8 and C18
MD0X014, MD0X015, MD0X009
Product Description

I. Intended Use

MagSi-proteomics beads are magnetic silica beads coated with C4, C8 or C18 groups, providing a reversed phase (RP) surface chemistry. The beads are an ideal tool for protein and peptide sample concentration, desalting and fractionation, and reducing sample complexity (Fig. 1). The different versions of MagSi-proteomics beads are intended for:

MagSi-proteomics C4:

- capture, concentration and purification of proteins from protein mixtures in general, cell lysates, culture supernatant (e.g. secreted proteins).

MagSi-proteomics C8:

- capture and purification of peptides and proteins from the following clinical samples: urine, saliva and CSF

MagSi-proteomics C18:

- Desalting of peptides or protein tryptic digest prior to mass spectrometry, concentration of peptides (e.g. secreted peptides into media), capture and purification of peptides and proteins from serum and plasma

Note: For **tissue samples** it is recommended to use **MagSi-WCX** or **MagSi-WAX**.

MagSi-proteomics C18 beads are an ideal tool for the purification, concentration and desalting of peptides and protein digests. MagSi-proteomics C8 beads represent an intermediate hydrophobicity (less hydrophobic than C18 and more hydrophobic than C4) and are suitable for sample preparation for proteomic profiling and biomarker research. The relatively low hydrophobicity of MagSi-proteomics C4 is most suitable for purification and fractionation of larger biomolecules like proteins. MagSi-proteomics beads are suitable for use in 96 well microplates on automated liquid handling platforms.

II. Principle

Peptides and proteins bind to MagSi-proteomics beads via hydrophobic interactions between the protein/peptide and the hydrophobic surface of the beads. The higher the hydrophobic character of the proteins and peptides the stronger the binding towards the reversed phase surface. Proteins and peptides are eluted under organic solvent conditions, e.g. acetonitrile (ACN). Proteins and peptides can therefore be separated according to their relative hydrophobicities using stepwise desorption in increasing concentrations of organic solvents.

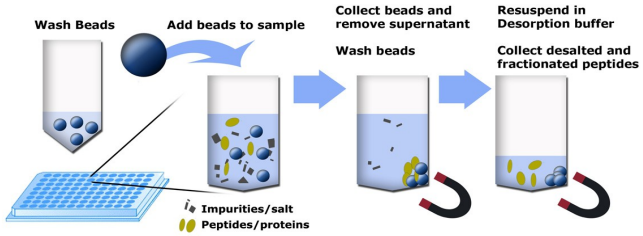


Fig. 1: Principle using MagSi-proteomics beads.

III. Material Supplied

- 2 or 10 mL MagSi-proteomics C4, MagSi-proteomics C8 or MagSi-proteomics C18 (supplied at 10 mg/mL in 25% ethanol absolute)

Additional materials needed:

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

- Mixer/vortex to mix samples and resuspend beads
- Magnetic separator for bead separation/collection (see order information)
- Solvents and reagents like ACN and TFA

Buffers and reagents needed:

We recommend to use the following buffers with the MagSi-proteomics beads and only use HPLC grade reagents.

- **Adsorption solution:** 0.1% trifluoroacetic acid (TFA), NaCl up to 200 mM can be added using MagSi-proteomics C4 and C8 beads
- **Washing solution**: 0.1% trifluoroacetic acid (TFA)
- **Desorption solution**: Typically 50% ACN in 0.1% TFA.

  Note: Fractionation of proteins/peptides is possible by using different concentrations of ACN (e.g. 20%, 50%, 80%)

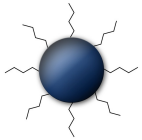
Use of detergents:

For better handling, detergents like 0.01% Tween 20 or 0.01% TX-100 can be used. However, please note that detergents may interfere with downstream applications like mass spectrometry. It is recommended to use up to 8 mM n-octylglucoside for **serum analysis.**

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 beads in well closed vial and in upright position to prevent drying of the beads. Do not freeze the product! Vortex bead suspension well before use.

MagSi-proteomics beads are compatible with common solvents used in mass spectrometry applications. No degradation or decrease of functionality could has been measured after incubation of beads in ACN concentrations up to 80%, several alcohols like MeOH or EtOH, and TFA up to 0.5%.



V. Protocols

Washing procedure:

1. Resuspend the beads
2. Transfer 20 µL to a tube
3. Place the tube on the magnet for 2 minutes.
4. Remove the supernatant by aspiration with a pipette while the tube remains on the magnet.
5. Remove the tube from the magnet.
6. Add 100 µL **Adsorption solution** and resuspend the beads.
7. Repeat steps 3 to 5 twice, for a total of three washes.
8. Add 10 µL adsorption solution and resuspend.

Peptide/protein adsorption:

1. Add your peptide sample to the vial containing the washed MagSi beads in **Adsorption solution**. Add TFA to a final concentration of 0.1% while adjusting the total volume to 25 µL. Mix using a pipette.
2. Leave at room temperature for 5-10 minutes to allow peptides/ proteins to adsorb to the beads.
3. Place the tube on the magnet. When the beads are at the tube wall and the liquid is clear, discard the supernatant.
4. Remove the tube from the magnet, add 50 µL **Washing Solution** and mix.
5. Separate the beads from the buffer using the magnet and discard the supernatant.
6. Repeat steps 4 and 5 twice, for a total of 3 washes.

Peptide/protein desorption:

1. Resuspend the beads in 10 µL **Desorption solution** and incubate for 5 – 8 minutes at room temperature.
2. Place the tube on the magnet and transfer the eluate containing the peptides or proteins to a new tube.

Sample analysis: Typically, 1 µL of the eluate and 1 µL of a saturated solution of a suitable MALDI-MS matrix is mixed (typically, alpha-cyano-4-hydroxy-cinnamic acid is used for peptides <4000 Da; for proteins >4000 Da, sinapinic acid is used). Spotting of 1 µL of the mixture on a MALDI target generates reliable spectra.

Troubleshooting:

Table 1: Possible problems and solutions for applications using MagSi-proteomics.

Problem	Suggested solutions
Poor adsorption of peptides and proteins	Use denaturing conditions during adsorption. Add guanidine HCL to the sample to achieve a final concentration between 1-6 M Use salt during adsorption to promote the hydrophobic interaction (up to 200 mM NaCl) especially using the MagSi-proteomics C4 beads
Difficulty eluting peptides / proteins from beads	Increase the acetonitrile concentration used during elution up to 80% or decrease the NaCl concentration used
Low elution volumes	Use larger samples volumes and optimize the bead to sample ratio by titration
Particles tend to stick to plastics	Add low concentration of polar solvents like isopropanol to the adsorption solution

VI. Technical Data

Table 2: Technical data for MagSi-proteomics C4, C8 and C18.

Product Name	MagSi-proteomics C4, C8 and C18
Mean size	1.2 µm
Supplied product volume	2 mL, 10 mL
Material	Magnetic silica particles with reversed phase chemistry on the surface (C4, C8 and C18).
Magnetic content	60%
Solution additives	25% ethanol in filtered demineralized water
Storage	Store at 2-8°C

VII. Additional Information

Disclaimer:

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

Order Information:

Product	Volume	Art.No.
MagSi-proteomics C4	2 mL	MD01014
MagSi-proteomics C4	10 mL	MD02014
MagSi-proteomics C8	2 mL	MD01015
MagSi-proteomics C8	10 mL	MD02015
MagSi-proteomics C18	2 mL	MD01009
MagSi-proteomics C18	10 mL	MD03009

Related products:

Product	Art.No.
MM-Separator M12+12 P	MDMG0001
MM-Separator M96 P	MDMG0002

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