

I. Intended use

MagSi-WCX beads are magnetic silica beads coated with a weak cation exchange surface (WCX). The beads are intended for:

- sample preparation and pre-fractionation prior to mass spectrometry (e.g. MALDI-TOF analysis) and HPLC
- protein and peptide separation for multiple downstream applications, e.g. enzymatic assays
- detergent removal
- Fractionation of clinical samples e.g. serum, plasma, tissues, CSF, urine and cell lysate

MagSi-WCX enables easy handling in both manual and automated workflows. The high magnetic strength of the beads typically results in complete collection in less than 1 minute when magnetic force is applied. Fast and complete separation results in very good reproducibility since no beads will be lost during washing steps. In addition, short incubation times for protein adsorption, desorption and magnetic collection typically significantly decreases the protocol time over conventional column based ion exchange chromatography, e.g. HPLC.

MagSi-WCX beads are suitable for use in 96 well microplates on automated liquid handling platforms.

II. Principle

Peptides and proteins bind to cationic groups on the surface of MagSi-WCX, while impurities are washed away (Fig. 1). After elution in Desorption buffer, purified peptides and proteins are ready for downstream use.

III. Material Supplied

- 2 or 10 ml MagSi-WCX (supplier at 20 mg/ml in filtered demineralized water)

Additional materials needed

- Magnetic separator for bead collection (see order information)
- Mixer/vortex to homogenize samples and re-suspend beads
- Buffer as outlined below, pipette tips and suitable tubes

Buffers and reagents needed

- **Pre-load solution:** Load WCX beads with counter ions: 0.05 M Ammonium Acetate (AmAc) pH 4 plus 1 M NaCl
- **Adsorption solution:** 0.05 M AmAc, pH 4
- **Washing solution:** water (HPLC grade)
- **Desorption solution 1** for MALDI MS: 1% TFA in water
- **Desorption solution 2:**
 - a) 0.05 M AmAc, pH 4, 0.2 M NaCl
 - b) 0.05 M AmAc, pH 4, 0.4 M NaCl
 - c) 0.05 M AmAc, pH 4, 0.6 M NaCl
 - d) 0.05 M AmAc, pH 4, 0.8 M NaCl
 - e) 0.05 M AmAc, pH 4, 1 M NaCl

For buffer systems, the pI of your target molecule should be taken into account. For efficient adsorption and desorption, the pH of the adsorption and desorption buffer should be at least one pH unit below the pI of the molecule to be bound, but should not exceed 4 pH units.

For analysing body fluids like serum, we recommend to test further buffer systems at different pH as well, since typically the pI of the target molecule(s) are unknown.

Optional adsorption and desorption buffers:

- 50 mM Sodium citrate, pH 5 (with increasing NaCl concentrations as for the AmAc system)
- 50 mM MES, pH 6
- 50 mM sodium phosphate pH
- 50 mM HEPES pH 8

Detergents:

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

IV. Product 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 re-suspend. Do not freeze the product! Vortex bead suspension well before use.

The beads are suspended in filtered water. This suspension media can easily be replaced with your own buffer/storage media.

The beads can be used in a pH range from 2,5 to 13. At these conditions, no degradation or iron oxide leakage was measurable using spectrophotometric assays. The beads are proofed to be compatible with mass spectrometry work-flows. Nevertheless, if you expect iron interference in downstream applications, we strongly advise you to rinse the beads before use.

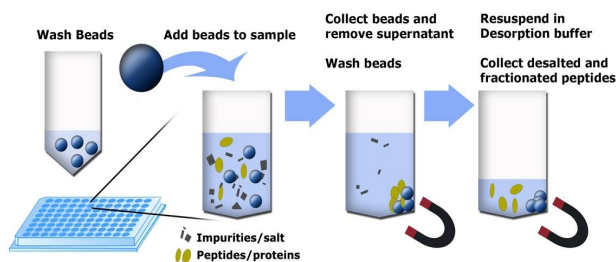


Fig. 1: Principle using MagSi-WCX beads.

V. Protocols

1) Pre-loading with Counter Ions:

1. Vortex MagSi-WCX beads to a homogeneous suspension.
2. Transfer 20 µl beads to an Eppendorf tube.
3. Place the tube to the magnet for 2 minutes.
4. Remove the supernatant.
5. Remove the tube from the magnet.
6. Add 200 µl **Pre-load Solution** and re suspend.
7. Place the tube to the magnet for 2 minutes, discard the supernatant
8. Repeat step 6 and 7 two times.

2) Equilibration to Adsorption buffer:

1. Add 200 µl **Adsorption Solution** to the bead pellet from 2.7 and re-suspend.
2. Magnetic separation for 2 min, discard the supernatant.
3. Wash the beads in **Adsorption Solution** 2x more.

3) Adsorption of Protein/Peptides:

1. Add your sample containing approx. 10 µg protein or peptide to the washed MagSi-WCX beads and add **Adsorption Solution** to a total volume 200 µl.
2. Leave the beads at room temperature for about 5 min. for proper adsorption of the sample. Continuous shaking is of advantage.
3. Place on the magnetic separator until the liquid is totally clear, discard the supernatant.
4. Remove the tube from the magnet and add 200 µl **Adsorption Solution**.
5. Magnetic separation for two minutes, discard the supernatant.
6. Repeat washing (steps 4-5) to a total of three times, discard the supernatant.

4) Desorption :

A) Desorption for MS Analysis:

1. Add 100 µl **Washing Solution** to the bead pellet and resuspend (desalting step)
2. Magnetic separation for 2 min., discard the supernatant.
3. Add 10 µl **Desorption Solution 1** to the beads, re-suspend
4. Magnetic separation for 2 min, remove the liquid for further analysis to a fresh Eppendorf tube.

MALDI analysis: Typically, 1 µl of the eluate and 1 µl of a saturated solution of a proper 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.

B) Desorption under native protein conditions:

1. Re-suspend the beads in the 20 µl **Desorption Solution 2a** (0.05M AmAc, pH 4, 0.2 M NaCl). And incubate for 2 min. at room temperature.
2. Separate the beads at the magnetic separator and transfer the supernatant
3. Repeat step 1) and 2) with increasing salt concentrations of the **Desorption Solution 2 b-e**

5) Desalting after 4B:

If a desalting is needed after desorption under native conditions (4B), e.g. for mass spec analysis, we recommend the **MagSi-proteomics C4, C8 or C18 beads (Art.No. MD01014, MD01015, MD01009)**.

VI. Technical Data

Product Name	MagSi-WCX
Mean size	1.2 µm (+/- 0.1 µm)
Concentration	20 mg/ml
Supplied product volume	2 ml, 10 ml, 100 ml
Material	Magnetic silica beads with fast magnetic separation characteristics; stay long time in suspension.
Solution additives	Filtered demineralized water
Storage	Store at 2-8°C

VII. Additional Information

Disclaimer

For R&D use only. Not for drug, household or other uses. Avoid contact with the suspension buffer. When disposing the suspension buffer, flush with large amounts of water. Material Safety Data Sheet (MSDS) is available on our website at www.amsbio.com.

Order Information

Product	Volume	Art.No.
MagSi-WCX	2 ml	MD01023
MagSi-WCX	10 ml	MD02023

As a complementary product we also recommend to consider MagSi-WAX bead in your application:

Product	Volume	Art.No.
MagSi-WAX	2 ml	MD01025
MagSi-WAX	10 ml	MD02025

Related products

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