MagSi-WAX
Product Description

Art.No.: MD0X025

magtivio

I. Intended Use

MagSi-WAX beads are magnetic silica beads with a weak anion exchange surface (WAX). The beads are intended for:

- sample preparation and pre-fractionation prior to mass spectrometry (e.g. MALDI-TOF analysis) & 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 lysates
- Enrichment of phosphorylated proteins and peptides

MagSi-WAX 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-WAX 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-WAX, while impurities are washed away (Fig. 1). After elution in Desorption buffer, purified peptides and proteins are ready for downstream use.

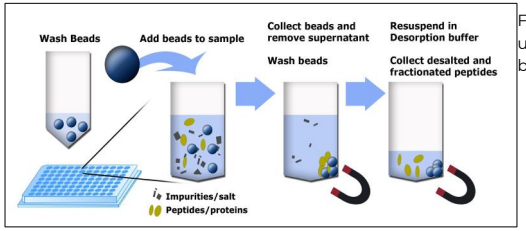

Fig. 1: Principle using MagSi-WAX beads.

III. Material Supplied

- 2 or 10 mL MagSi-WAX (supplied at 20 mg/mL in filtered demineralized water)

Additional materials needed

- Magnetic separator for bead separation/collecting (see ordering information)
- Mixer/vortex to homogenize samples and resuspend beads
- Buffer as outlined below, pipette tips and suitable tubes

Buffers and reagents needed

- **Pre-Load Solution**: Load MagSi-WAX beads with counter ions: 0.02 M bis Tris pH 6 plus 1 M NaCl
- **Adsorption Solution**: 0.02 M Bis-Tris, pH 6
- **Washing Solution**: water (HPLC grade)
- **Desorption Solution 1** for MALDI MS: 1% TFA in water
- **Desorption Solution 2**:

  a) 0.02 M Bis-Tris, pH 6, 0.05 M NaCl

  b) 0.02 M Bis-Tris, pH 6, 0.1 M NaCl

  c) 0.02 M Bis-Tris, pH 6, 0.15 M NaCl

  d) 0.02 M Bis-Tris, pH 6, 0.20 M NaCl

  e) 0.02 M Bis-Tris, pH 6, 0.25 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 buffers

- 0.1 % TFA (pH < 3.0)
- sodium citrate buffer, pH 3.5 – 4.5
- N-Methylpiperazine, 20 mM, pH 4.5 – 5.0
- Piperazine, 20 mM, pH 5.0 – 6.0
- Bis-Tris, 20 mM, pH 5.8 – 6.4
- Bis-Tris propane, 20 mM, pH 6.4 – 7.3
- Triethanolamine, 20 mM, pH 7.3 – 7.7

For the corresponding desorption solutions (salt step gradient), 0.05 M, 0.1 M, 0.15 M, 0.2 M and 0.25 M NaCl has to be added.

Detergents

For better handling detergents like 0.01 % Tween20 or 0.01 % Triton X-100 may be used. However, please note that detergents might 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 for 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 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 demineralized 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. The beads are proofed to be compatible with mass spectrometry workflows. Nevertheless, if you expect iron interference in downstream applications, we strongly advise you to rinse the beads before use.

V. Protocols

1) Pre-loading with Counter Ions

1. Vortex MagSi-WAX beads to a homogeneous suspension.
2. Transfer 20 µL slurry to a microtube.
3. Place the tube to the magnet for 1-2 minutes.
4. Remove the supernatant
5. Remove the tube from the magnet
6. Add 200 µL **Pre-Load Solution** and re-suspend
7. Magnetic separation 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 2.8. and resuspend
2. Magnetic separation for 2 min, discard the supernatant
3. Wash the beads in **Adsorption Solution** 2 more times

3) Adsorption of Protein/Peptides

1. Add your sample containing approx. 10 µg protein or peptide to the washed MagSi-WAX beads and add **Adsorption Solution** to a total volume of 100 µL.
2. Leave the beads at room temperature for about 5 min. for proper adsorption of the sample. Continuous shaking is of advantage.
3. Magnetic separation 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 steps 4-5 two times for a total of 3 washes, discard the supernatant.

4) Desorption

A) For MS analysis:

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

Sample 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** (0.02M Tris, pH 6, 0.05 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 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-WAX
Mean size	1.2 µm
Supplied product volume	2 mL, 10 mL, 100 mL
Material	Magnetic silica beads with weak cation exchange surface
Magnetic content	60%
Solution additives	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. Safety Data Sheet (SDS) is available at www.magtivio.com.

Ordering Information

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

Related products

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

Product	Art.No.
MM-Separator M12 + 12	MD90001
MM-Separator M96	MD90002
MM-Separator 96 PCR	MDMG0005