Content

1. Overview

1.1 MagSi benefits in the proteomics workflow.

MagSi silica particles with C4, C8 and C18 modified surface allows for the purification and fractionation of proteins and peptides for mass spectrometry, proteomic profiling and biomarker research The MagSi beads for proteomics applications are an alternative to on- or offline SPE columns, cartridges or plates used in biological specimens sample preparations. Key markets are clinical, medical and pharmaceutical research and diagnostics.

Proteins and peptides from complex (clinical) samples like serum, plasma, urine, saliva or cell lysates are bound to magnetic beads, fractionated based on their physical properties and finally eluted from the magnetic beads for further fractionation and read-out.

The result is a fractionated, cleaned up sample; removal of interfering salts and typically an enrichment of the target compounds.

Typically mass spectrometry, namely MALDI-TOF (Matrix assisted laser desorption time of flight) and ESI-TOF (Electro-spray ionization time of flight), is used as read-out for protein and peptide analysis. Additional fractionation is typically performed by capillary electrophoresis (CE).

Key advantage of magnetic beads over alternative SPE formats is the easy automation of the sample preparation process. No centrifugation or negative/positive pressure is needed in an automated sample preparation process. Therefore magnetic beads are ideally suited for high throughput applications.

Key advantages:

- Reproducible concentration and fractionation of your protein and peptide sample
- Reduction of sample complexity
- Easy removal of unwanted salts and detergents, also in parallel mode
- Highly suitable for high throughput applications and MTP format
- No centrifugation steps
- Compatible with small amounts and small volume samples
- Compatible with viscous samples

In proteomics applications magnetic particles are used as sample preparation tool to reduce either the complexity of a biological sample or as clean-up tool prior to a detection read-out.

Fractionation include:

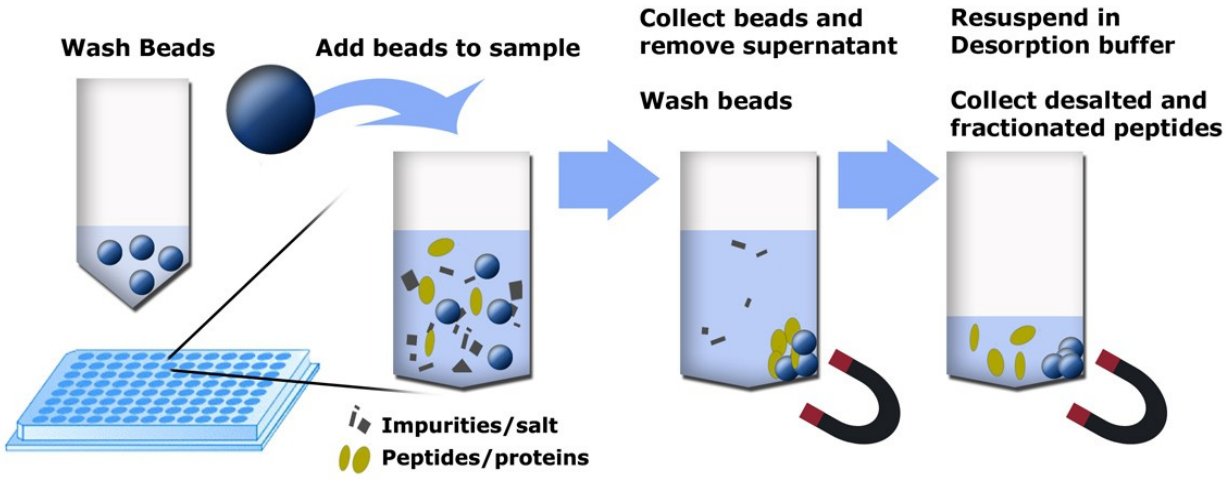
- one and two-dimensional gel electrophoresis
- capillary electrophoresis

Detection read-out include:

- mass spectrometry (most commonly used: MALDI-TOF and ESI-TOF)
- Gel-electrophoresis
- Immunoassay reader
- Fluorescence/Chemiluminescence detection

Sample preparation

Clean-up, concentration and enrichment of the analytical target. **The analytical target is bound to the magnetic beads,** while unwanted compounds, especially salts, are washed away**.**



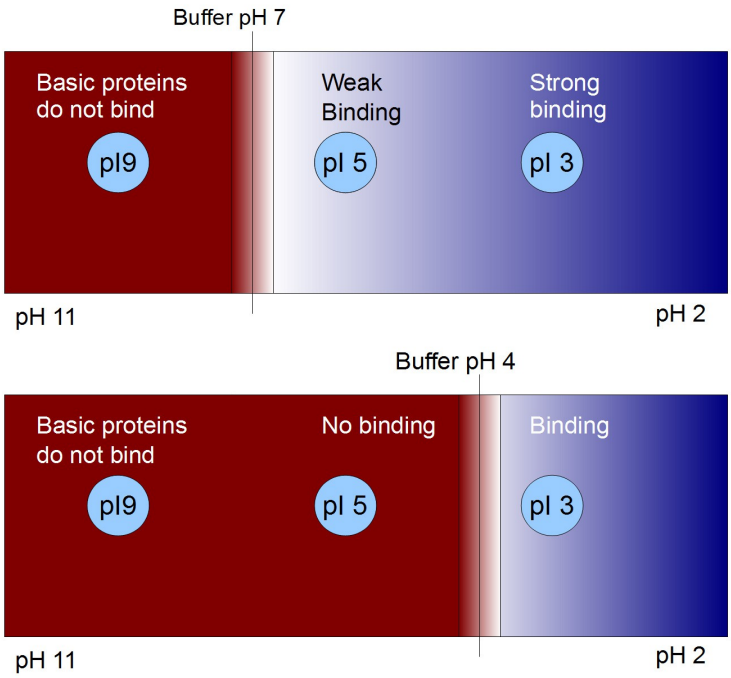
The discrimination between analytical targets and unwanted compounds is based on the physical properties of the compounds. Principle physical properties include: A) charge; B) hydrophobicity and C) size.

2. Selection based physical properties

2.1 Weak ion exchange magnetic beads MagSi-WAX and MagSi-WCX magnetic beads: Selection based on target surface charge

MagSi-WAX

Proteins and peptides bind to the MagSi-WAX beads based on the surface charge of the target proteins and peptides. Alkaline proteins, with an isolelectrical point > 7 (pI > 7) do not bind to the MagSi-WAX beads (using a buffer system at physiological pH) whereas acidic proteins (pI < 7) and peptides bind tightly to the MagSi-WAX beads. Bound proteins can be eluted from the MagSi-WAX beads either under high salt conditions or shift of the buffer pH towards more acidic conditions. The principle of the use of MagSi-WAX beads is outlined in the graphic below.

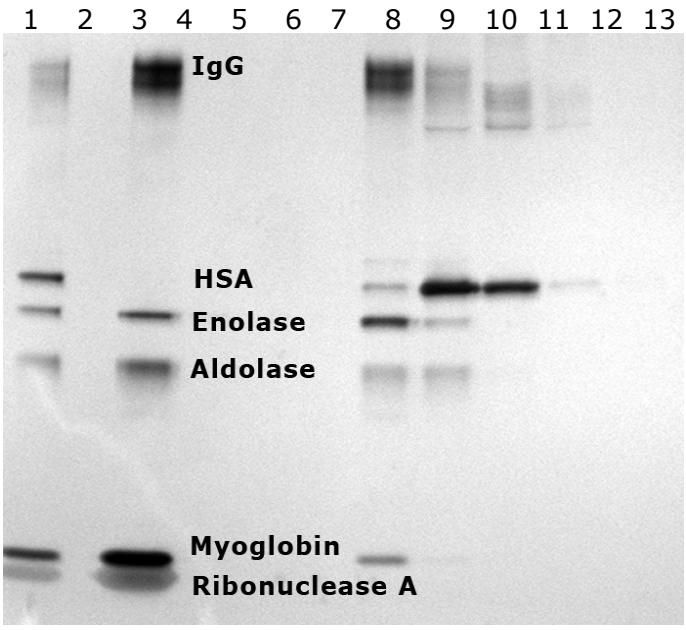


Graphic 1: Graphic view how basic, neutral and acidic proteins bind to weak anion exchange MagSi-WAX magnetic particles, depending on the pH of the working buffer system. If a neutral or basic buffer is used as working buffer (upper case) neutral and acidic proteins will bind to the MagSi-WAX magnetic particles whereas basic proteins will not bind at all (they can be collected in the flow through fraction for further separation by MagSi-WCX beads). The strength of the binding and hence fractionation within a salt gradient is depending on the individual pI of the proteins. The opposite is true for weak cation exchange MagSi-WCX beads (not shown). Basic and neutral proteins will be bound and fractionated according to the individual pI whereas acidic proteins will not bind and will be found in the flow through fractions.

The fractionation potential for a protein mix is demonstrated in figure 1. The following protein mix (10 mg/ml each) has been used:

Protein	Size	pI
Ribonuclease A	13,7 kDa	9,6
Myoglobin	17 kDa	6,8
Aldolase	39 kDa	7
Enolase	47 kDa	6,4
Albumin (HSA)	66 kDa	4,7
Human IgG	150 kDa	6

A



B

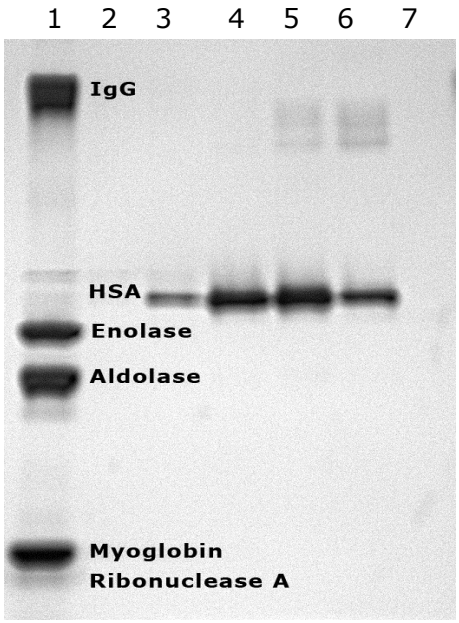


Fig. 1A) buffer pH 8.5 (Tris/HCl, 25 mM, pH 8.5): lane 1: protein standard; lane 2: free; lane 3: flow through (FT) fraction; lane 4: wash fraction 1; lane 5: wash fraction 2; lane 6: wash fraction 3; lane 8: elution with Tris/HCl, 25 mM, pH 8.5 plus 0.2 M NaCl; lane 9: elution with 0.4 M NaCl, lane 10: elution with 0.6 M NaCl; lane 11: elution with 0.8 M NaCl and lane 12: elution with 1.0 M NaCl. Fig. 1B): buffer pH 8.0 (Tris/HCl, 25 mM, pH 8): lane 1: flow through fraction, lane 2: wash fraction 1 (fraction 2 +3 not shown), lane 3: elution with 0.2 M NaCl, lane 4: elution with 0.4 M NaCl, lane 5: elution with 0.6 M NaCl, lane 6: elution with 0.8 M NaCl and lane 7: elution with 1.0 M NaCl.

Shifting the pH from pH 8.5 1A) to pH 8.0 1B) protein with slightly acidic pI like Enolase, Aldolase and Myoglobin are pushed from the salt gradient fractions in the flow through fractions, where as the more acidic human serum albumin was kept in the salt gradient according to graphic 1.

MagSi-WCX

The MagSi-WCX (weak cation exchange) magnetic beads are the "counter" beads to the MagSi-WAX beads. Binding, washing and elution are also triggered by the protein/peptide total net charge, but in the opposite way compared to the MagSi-WAX beads. Under physiological conditions proteins with alcaline isoelectrical point (pI > 7) are bound the MagSi-WCX beads whereas acidic proteins are found in the flow through fraction.

Since the MagSi-WCX and MagSi-WAX act as counter-beads, magtivio recommends to test both types of beads, to see which best suits your application.

2.2 Reversed phase magnetic beads MagSi-proteomics C4, C8 and C18, selection based on target hydrophobicity

MagSi-proteomics magnetic beads, containing C4, C8 or C18 alkyl groups on the surface, bind proteins and peptides under polar, aqueous conditions and elute the proteins or peptides under less/non-polar conditions using organic agents (typically acetonitrile). This Proteins and peptides can be fractioned using a step-wise increase of the organics/water ratio as demonstrated in figure 2.

A

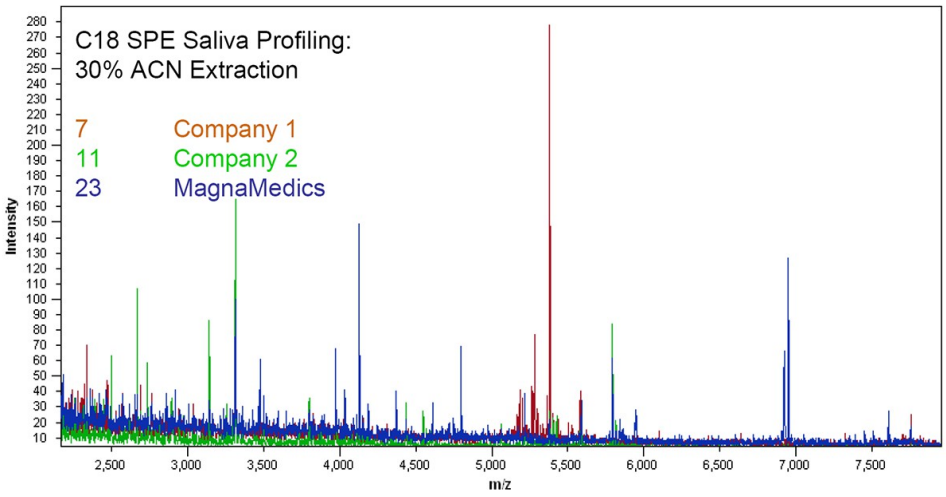


Figure 2A: Spectra of saliva sample peptides after elution with 30% acetonitrile.
MagnaMedics C18 beads (currently magtivio) show 23 annotated proteins (blue) as compared to the two reference beads, 7 (red) and 11 (green) respectively.

B

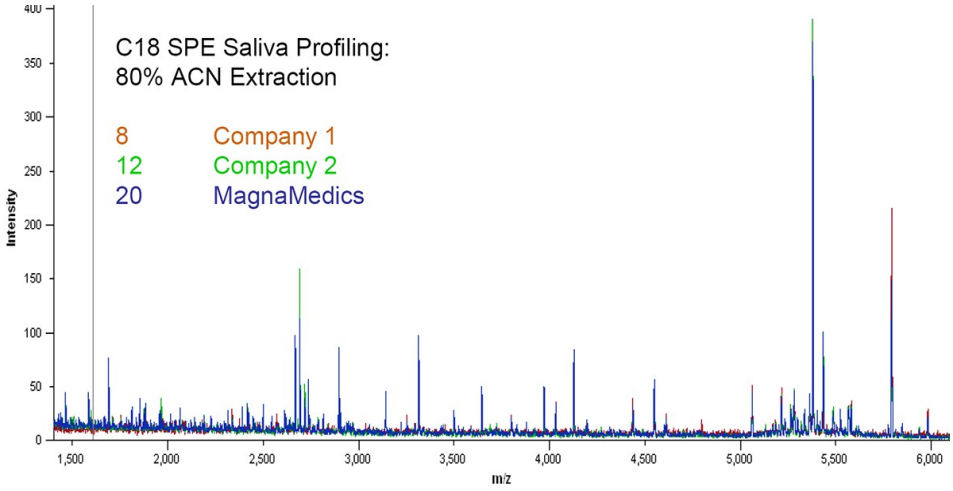


Figure 2B: Spectra of peptides after elution with 80% acetonitrile.The peptide patterns between 30% and 80% acetonitrile extraction is totally distinct.
MagnaMedics C18 beads (currently magtivio) show 20 annotated proteins (blue) as compared to the two reference beads, 8 (red) and 12 (green) respectively.

Native proteins have a hydrophobic core and a relative hydrophilic surface to keep the proteins fixed in

their 3-dimensional structure under aqueous conditions as in living cells and body fluids like serum or plasma. Therefore for protein fractionation MagSi-proteomics C4 beads are recommended over MagSi-proteomics C18 for protein analysis applications. The opposite is true for peptide analytic applications, where MagSi-proteomics C18 give best fractionation power.

2.3 Selection based on application

Denaturing conditions

e.g. mass spectrometry and gel electrophoresis

	MagSi-proteomics C4	MagSi-proteomics C8	MagSi-proteomics C18	MagSi-WCX	MagSi-WAX
Protein analysis (> 5 kDa) – denatured conditions**	++	+	-	-	-
Desalting protein samples**	++	+	-	-	-
Peptide analysis (< 5kDa)**	+	+	++	+	+
Analysis of single tryptic digests**	-	+	++	+	+
Desalting of tryptic digest samples**	-	+	++	-	-
Peptide mass fingerprinting**	+	+	++	+	+
Protein concentration**	++	+	-	-	-
Small molecule, peptide enrichment and clean-up (neutrally charged)	-	+	++	-	-
Clinical samples: Serum, Plasma, Urine, Saliva**	-	++	-	-	-
Acidic protein/peptide samples	+	+	+	-	++
Phosphoproteins	-	-	-	-	+
Metabolite analysis (hydrophobic, uncharged target)	-	+	++	-	-
Metabolite analysis (positively charged target)	-	-	-	++	-
Metabolite analysis (negatively charged target)	-	-	-	-	++
Basic and phys. pH protein/peptide samples	+	+	+	++	-

*++ best suited and recommended by magtivio; + suitable but not optimal; - not recommended under physiological conditions; **specific literature available as application note;*

Native conditions

e.g. bio-chromatography, enzyme activity, protein-protein interactions and crystallography.

	MagSi-proteomics C4	MagSi-proteomics C8	MagSi-proteomics C18	MagSi-WCX	MagSi-WAX
Acidic proteins	-	-	-	-	++
Basic proteins	-	-	-	++	-
Phosphoproteins	-	-	-	-	++

2.4 Depletion of unwanted compounds – Reduction of matrix effects

Biological samples are complex mixtures containing a target of interest and many other compounds which may have a negative impact on the read-out. These unwanted compounds, also called matrix, needs to be eliminated within the sample preparation process.

Typically solid phase extraction solutions, based on columns, tips and cartridges are used for this approach. Thereby the analytical target is bound to the solid phase extraction (SPE) chromatographic material, impurities are washed away and the target is eluted from the SPE material.

Alternatively, MagSi beads for proteomics applications can be used to deplete unwanted compounds, thereby eliminate matrix effects and keeping the cleaned-up analytical target in suspension for further down-stream analysis. The results is a cleaned-up sample, where matrix effect causing compounds are eliminated or significantly reduced.

Key advantages of this process are:

- Elimination of matrix effects, especially phopholipids
- Fully automatable
- High throughput, parallel processing
- Scalable for very small and very large sample quantities
- Very fast and easy protocols saving valuable human resources
- Multiple combinations possible for extended clean-up

For the depletion of proteins as interfering matrix compound we refer to the MagSiMUS products and the MagSiMUS selection guide.

Depletion of unwanted compounds

	MagSi-proteomics C4	MagSi-proteomics C8	MagSi-proteomics C18	MagSi-WCX	MagSi-WAX	MagSi-Lipid^{CTRL}
Depletion of lipids	-	+	++	-	-	-
Depletion of phospholipids	-	-	-	-	-	++

Depletion of Phospholipids (under development)

For the depletion of phospholipids magtivio develops the MagSi-Lipid^{CTRL} magnetic beads. MagSi-Lipid^{CTRL} can be used in combination with MagSiMUS-TDMPREP and MagSiMUS-TOXPREP products as additional clean up especially for removal of ion-suppression causing phospholipids.

3. Description of the MagSi beads used in proteomics applications

3.1 MagSi-proteomics reversed phase magnetic beads (C4, C8, C18)

MagSi-proteomics beads are magnetic beads that are an ideal tool for the purification, concentration and desalting of peptides and protein digests. The surface of the beads has been modified with C4, C8 and C18 -alkyl groups that are typical for reversed phase applications.

MagSi proteomics reversed phase beads have several advantages:

- Magnetic beads can be used in automated liquid handling platform in high throughput mode
- Significantly enhances the throughput compared to the widely used tip based solutions
- Up to 3 times more cost effective compared to ZipTips™

The MagSi-proteomics beads have been demonstrated as powerful tool in desalting of proteomics sample after protein digestion and prior to mass spectrometry.
MagSi-proteomics efficiently bind and elute even tiny sample fractions. Peptide amounts as low as 20 – 50 ng can be desalted by MagSi proteomics C8 and C18 beads and analyzed by state of the art MALDI-TOF instruments.

3.2 MagSi beads for serum, plasma, urine, saliva and other body fluids.

In highly automated workflows, using state of the art liquid handling systems, we highly recommend to consider MagSi-proteomics C8 beads besides of the C18 beads. The MagSi proteomics C8 beads are less hydrophobic and therefore do not tend to stick to plastic ware as outlined by one of our clients:

"The MagSi C8 beads worked very well with diluted saliva in comparison with another (non Dynal) magnetic surface. There were more peaks detected with the MagSi beads and with higher intensity. They were pulled down much more easily than the MagSi C18 beads, did not stick to the well sides, and were not lost during washes. They also appeared to have a greater binding capacity than the competitor as increasing the amount of saliva in the binding reaction led to higher and higher signal while the other C8 bead saturated at a lower concentration of sample" (James LeBlanc, UCLA)

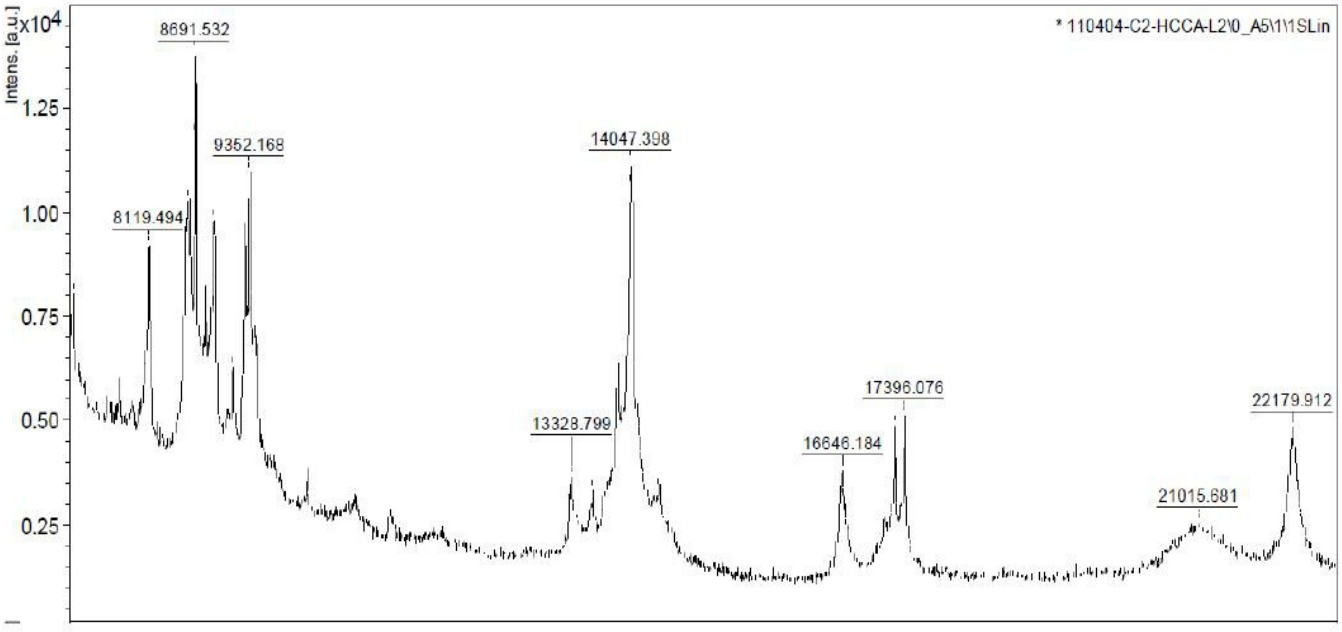


Figure 2: 20 µl of serum sample purified using MagSi-proteomics C8 magnetic particles analysed on MALDI-TOF

The binding capacity for the MagSi-proteomics C8 beads is found to be higher (20 µg/mg beads) compared to MagSi-proteomics C18 (15 µg/mg beads). MagSi-proteomics C8 beads are easily scalable, no saturation effects were observed compared to competitor beads. Therefore, magtivio clearly recommends to consider the MagSi proteomics C8 beads in profiling peptide patterns form any body fluid like serum, plasma, urine or saliva.

4. Catalogue information

4.1 MagSi-proteomics

MagSi-proteomics beads are magnetic beads that are an ideal tool for the purification, concentration and desalting of peptides and protein digests. The surface of the beads has been modified with C4, C8 and C18 -alkyl groups that are typical for reversed phase applications.

MagSi-proteomics order information

Art. Nr.	Product	Conc.	Size	Volume
MD01014	MagSi-proteomics C4	10 mg/ml	1.2 µm	2 ml
MD02014	MagSi-proteomics C4	10 mg/ml	1.2 µm	10 ml
MD01015	MagSi-proteomics C8	10 mg/ml	1.2 µm	2 ml
MD02015	MagSi-proteomics C8	10 mg/ml	1.2 µm	10 ml
MD01009	MagSi-proteomics C18	10 mg/ml	1.2 µm	2 ml
MD03009	MagSi-proteomics C18	10 mg/ml	1.2 µm	10 ml

4.2 MagSi-WCX

The MagSi-WCX (weak cation exchange) magnetic beads have the typical ion exchange properties well known from classical chromatography. Protein and peptides are charged at the surface and will be adsorbed to magnetic beads under low salt condition. Under high salt conditions or by pH shift the target proteins/peptides are eluted (ion exchange). If you expect more analytes with acidic pI we recommend to consider also the MagSi-WAX beads (below).

The high magnetic strength makes them applicable for both manual and automated/robotic fractionation, because the beads will typically collect in less than 1 minute when magnetic force is applied. This quick and complete separation gives very good reproducibility since no beads will be lost during washing steps. Furthermore the quick protein adsorption, desorption and magnetic collection typically shortens significantly the protocol time over conventional column-based ion exchange chromatography.

MagSi-WCX order information

Art. Nr.	Product	Conc.	Size	Volume
MD01023	MagSi-WCX	20 mg/ml	1.2 µm	2ml
MD02023	MagSi-WCX	20 mg/ml	1.2 µm	10ml

4.3 MagSi-WAX

The MagSi-WAX (weak anion exchange) magnetic beads are the "counter" beads to the MagSi-WCX beads. Binding, washing and elution are also triggered by the protein/peptide total net charge.
The MagSi-WAX beads are ideally suited when more acidic proteins/peptides are expected in the sample. However, our protocols given in the corresponding product sheet cover buffer systems for acidic, neutral and basic conditions.
Since the MagSi-WCX and MagSi-WAX act as counter-beads, magtivio recommends to test both types of beads, to see which best suits your applications.

MagSi-WAX order information

Art. Nr.	Product	Conc.	Size	Volume
MD01025	MagSi-WAX	20 mg/ml	1.2 µm	2 ml
MD02025	MagSi-WAX	20 mg/ml	1.2 µm	10 ml

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