CONSUMABLES AND SUPPLIES

LIFE SCIENCE

- Proteomics ··· 55~82

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  EVSecond

Phosphorylation Purification & Enrichment

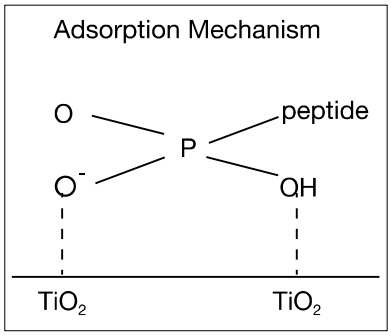
Phosphorylated Protein Research

Protein phosphorylation is recognized as a fundamental process which regulates cell differentiation, growth, and migration. However, in protein phosphorylation analysis, the low concentration of any given phosphoprotein may result in low ionization efficiency and the detection by MS may be affected. Therefore, enrichment of phosphopeptides and the relative reduction of non-phosphorylated peptides is critical to accurate analysis of protein digests by LC/MS.

GL Sciences' Titanium Dioxide (TiO_2 or Titania) products have emerged as the most effect means of phosphopeptide enrichment of protein digests prior to LC/MS analysis, replacing IMAC as the primary means of phosphopeptide sample pretreatment. Enrichment by titanium dioxide and IMAC, remain, however, complimentary techniques and are often used in combination to obtain optimal phosphopeptide analysis.

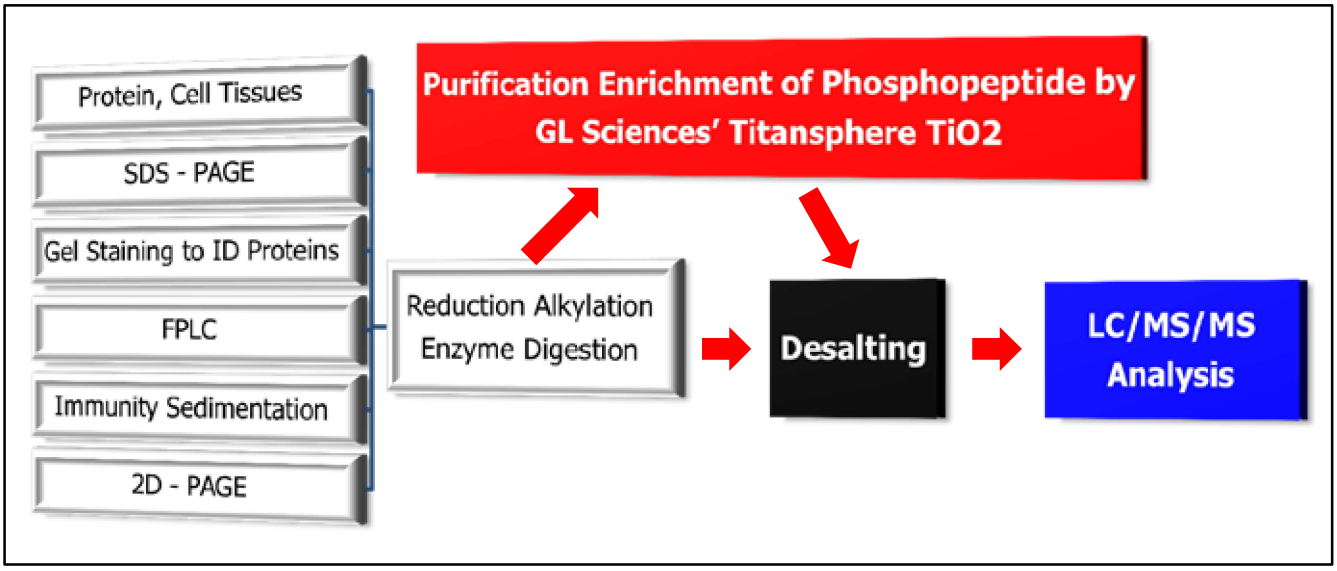
What Makes GL Sciences' Titanium Dioxide Products Unique and Superior?
Titanium Dioxide exists in three crystaline forms, known as rutile, anatase, and brookite. Rutile and Anatase forms are the most common and most useful for phosphopeptide enrichment, and the ratio of rutile form to anatase form has significant implications for applicabililty to enrichment of phosphopeptides. Due to its unique manufacturing technique, GL Sciences can produce phosphopeptide enrichment products with highly spherical beads having the optimum ratio of crystal forms of TiO_2. The primary reasons the GL Sciences' Pho-TiO products show superior performance is a direct result of the unique formulation of our titanium dioxide beads.

Principal of Phosphopeptide Enrichment



Phosphate groups are preferentially adsorbed to the surface of titanium dioxide under acidic condtions and are eluted under basic condition. Non-phosphorylated acid peptides non-specifically bound to the TiO_2 can be reduced by adding acid modifiers to the loading and/or wash buffers.

Basics of Phosphopeptide Analyses by LC/MS/MS



Titansphere Bulk Materials

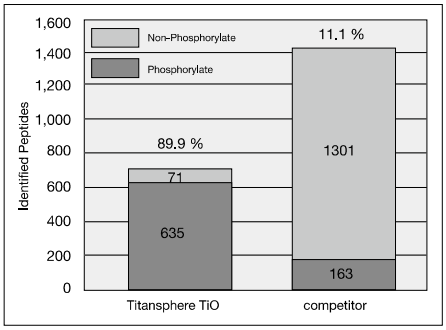
Bulk Sorbent Materials for Purification & Enrichment of Phosphopeptides

While GL Sciences' Phos-TiO spin columns based enrichment products are useful for most sample pretreatment applications, some investigators require bulk titanium dioxide media for specific applications. Our market leading Titansphere Phos-TiO Kit is now available in bulk media which is Titansphere Phos-TiO 10 µm bulk media, and is optimized for purifying and enriching more phosphopeptide.

Applications

Efficient Purification from HeLa Cell Lysate

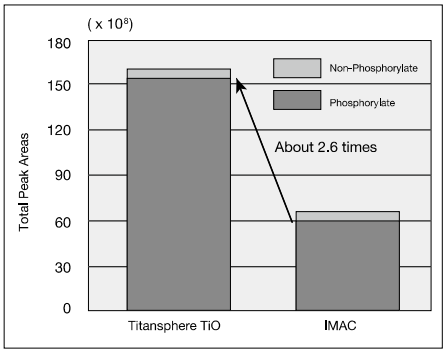
The data on the right shows the superior performance of Titansphere TiO over another competitor brand. HeLa Cell Lysate which consists mainly of non-phosphorylated peptides, was used as sample. Titansphere TiO shows exceptional selectivity - almost 90 % of the bound peptides were phosphopeptides, and excellent capacity for total phosphopeptide binding. While the competitive brand shows more selectivity for non-posphorelated peptides.



Sample : HeLa Cell Lysate, Sample volume : 50 µg,
Titansphere TiO volume : 1 mg

Compare Titansphere TiO with IMAC

The graph on the right shows the difference between Titansphere TiO and the normal IMAC enrichment method. Using Arabidopsis cell extract. Titansphere TiO provides substantially higher total peak area and a much higher number of non-phosphorelated peptides.



Sample : Arabidopsis Cell Extract, Sample volume : 100 µg,
Titansphere TiO volume : 1 mg

Specifications

Description	Titansphere TiO
Particle Size	5 µm, 10 µm
Particle Shape	Spherical
Adsorption Spot	Titanium Dioxide Crystal
Pore Size	100 Å (10 µm)
pH Range	2–12
Gravity	1.74

Titansphere Bulk Materials (Previous Version)

Description	Particle Size	Qty.	Cat.No.
Titansphere TiO 5 µm, 500 mg	5 µm	1/pk	5020-75000
Titansphere TiO 10 µm, 500 mg	10 µm	1/pk	5020-75010

Titansphere Phos-TiO Bulk Material

Bulk Sorbent Materials for Purification & Enrichment of Phosphopeptides

While GL Sciences' Phos-TiO spin tips based enrichment products are useful for most sample pretreatment applications, some investigators require bulk titanium dioxide media for specific applications. A new bulk material is now available: Titansphere Phos-TiO is the new version of Titansphere TiO designed for even better absorption capacity of phosphopetides.

References

1. Phosphopeptide enrichment by aliphatic hydroxy acid-modified metal oxide chromatography for nano-LC-MS/MS in proteomics applications, Sugiyama N, Masuda T, Shinoda K, Nakamura A, Tomita M, Ishihama Y., Mol Cell Proteomics. 2007 Jun;6 (6) : 1103-9.
2. Highly selective enrichment of phosphorylated peptides using titanium dioxide, Nature Protocols 1, 2006, 1929-1935.
3. Global, in vivo, and site-specific phosphorylation dynamics in signaling networks, Olsen JV, Blagoev B, Gnad F, Macek B, Kumar C, Mortensen P, Mann M., Cell. 2006 Nov 3;127 (3) : 635-48.
4. Successive and selective release of phospholylated Peptides captured by Hydroxy Acid-Modified Metal Oxide Chromatography, Yutaka Kyono, Naoyuki Sugiyama, Koshi Imami, Masaru Tomita, and Yasushi Ishihama, J Proteome Res., 2008, 7 (10), 4585-93.
5. Extended coverage of singly and multiply phosphorylated peptides from a single Titanium Dioxide Microcolumn, Anal Chem., 2015, 87 (20), 10213-21.

Titansphere Phos-TiO Bulk Material (New Version)

Description	Particle Size	Qty.	Cat.No.
Titansphere Phos-TiO Bulk 10 µm, 500 mg	10 µm	1 pc	5010-21315

Titansphere Phos-TiO MP Kit

Efficient Enrichment of BOTH Singly and Multiply Phosphorylated Peptides

GL Sciences is widely recognized as the best manufacturer for the enrichment/purification of phosphopeptide, Titansphere Phos-TiO kit and bulk resins are widely used throughout the world in major cancer research institutes and proteomics core facilities.

The new Titansphere Phos-TiO MP Kit employs a new protocol in the HAMMOC method, which enables highly efficient and selective recovery of not only singly, but also multiply phosphorylated peptides. Specifically, with the new kit it is possible to fraction the singly and the multiply phosphorylated peptides separately, preventing ion suppression in LCMS/MS detection and delivering higher recovery of multiply phosphorylated peptides.

Features

- High Recovery of not only Singly, but also Multiply Phosphorylated Peptides.
- All operation is done using an easy-to-use centrifuge.

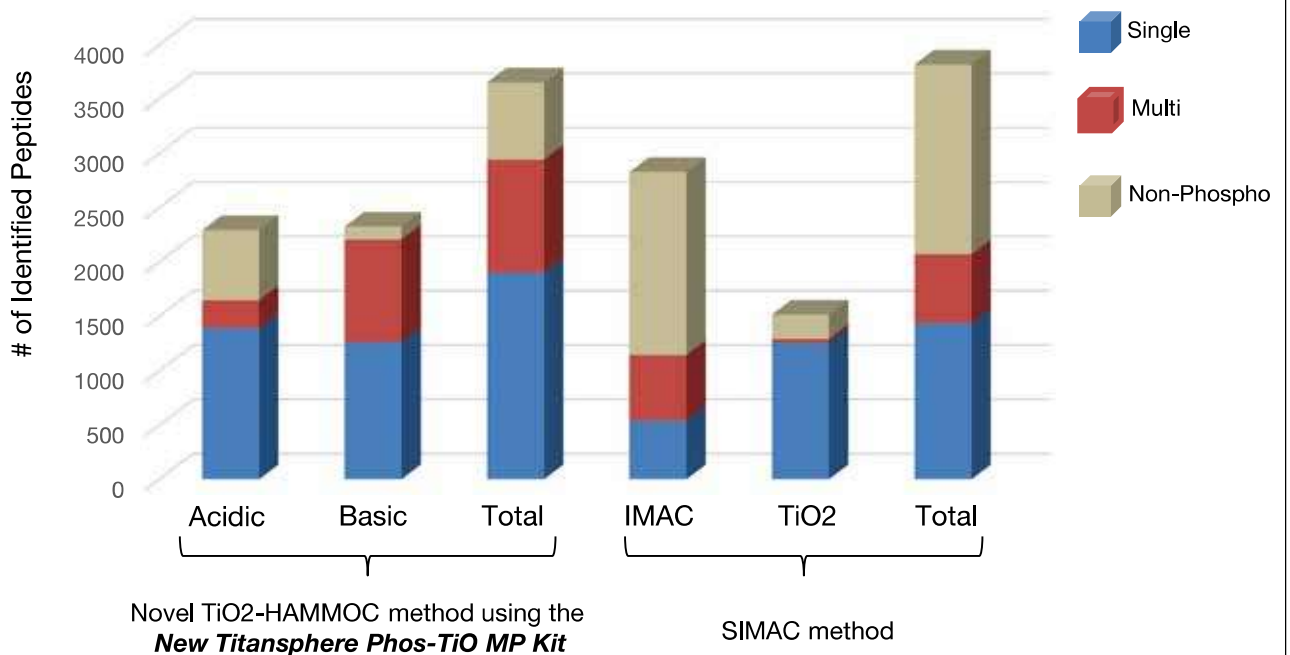
Sample Loading Capacity

Description	Content	
Sample	Tyr (PO_3H_2) - Angiotensin II	
Spin Tip Sorbent Mass/Tip Volume	1 mg/200 µL	3 mg/200 µL
Sample Loading Capacity	1.2 µg	3.5 µg

Titansphere Phos-TiO MP Kit Contents

Cat.No.	5010-21282	5010-21283
Titansphere Phos-TiO MP Kit	24 pcs	24 pcs
Titansphere Sorbent Mass/Tip Volume	1 mg/200 µL	3 mg/200 µL
Spin Tip Quantity	24 pcs (6 x 4 packs)	24 pcs (6 x 4 packs)
Waste Fluid Tube Quantity	24 pcs	24 pcs
Recovery Tube (2.0 mL) Quantity	24 pcs	48 pcs
Recovery Tube (1.5 mL) Quantity	48 pcs	48 pcs
Solution B (Lactic acid) Quantity	2 mL	2 mL
Instruction Manual	1/pk	1/pk

Comparison of Recovery of BOTH Singly and Multiply Phosphorylated Peptides



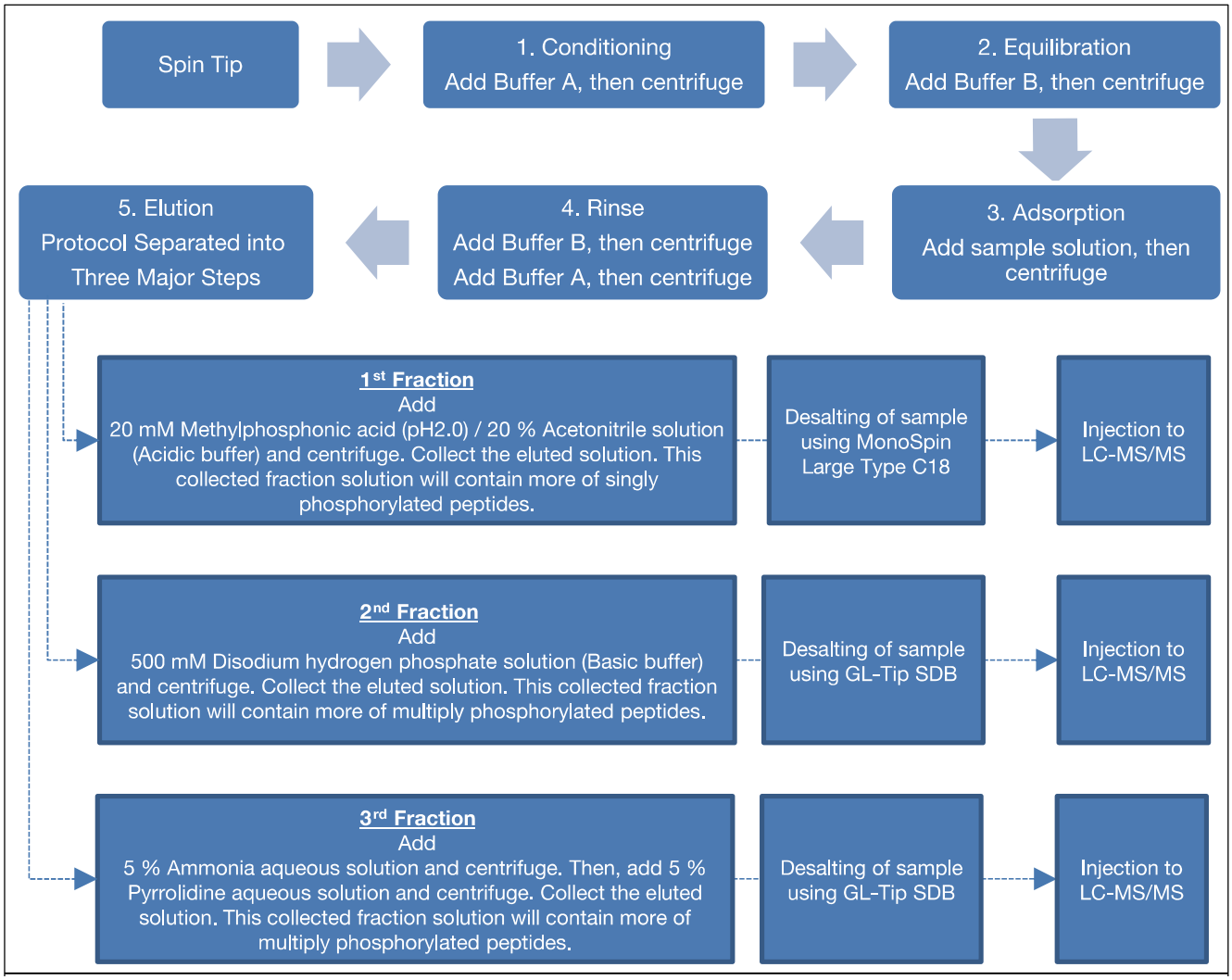
Data from Wakabayashi et al. Ana Chem 2015, 87

The above experiment was done using HeLa cells 100 µg to test the new Titansphere Phos-TiO MP Kit which showed highly efficient recovery of both singly and multiply phosphorylated peptides compared to SIMAC (Sequential Elution from IMAC) method.

The SIMAC method generally consists of into two major protocols, in the first one the Immobilized Metal Ion Affinity Chromatography is used to make the first enrichment and separation of mono and multi-phosphorelated peptides; and in the second one a further enrichment of the mono-phosphorelated peptides is carried out using titanium dioxide chromatography. Finally, the two separated phosphopeptide fractions are analyzed by LC-MS/MS.

In the new Titansphere Phos-TiO MP Kit, more singly phosphorylated peptides were recovered more when using an acidic elution buffer, while the more multiply phosphorylated peptides were recovered more when using a basic elution buffer. As proven above, in total, the new Titansphere Phos-TiO MP Kit is able to recover more both singly and multiply phosphorelated peptides than the SIMAC method.

Typical Enrichment Protocol using Titansphere Phos-TiO MP Kit



Remarks

- Buffer A, Buffer B, Methylphosphonic acid, Disodium hydrogen phosphate, Ammonia aqueous solution and 5 % Pyrrolidine aqueous solution are not included in the kit due to their designation as hazardous materials for the purposes of air transportation. Therefore, the users must prepare these solutions in their lab, according to the procedure described in the instruction manual.
- Solution B is included in the kit, which is Lactic acid.
- The preparation procedure of Buffer A and B are as follows.

Buffer A	2 % TFA solution	1 mL
	Acetonitrile	4 mL
	Total	5 mL (Use 3 mL for making Buffer B)

Buffer B	Solution B	1 mL
	Buffer A	3 mL
	Total	4 mL

Titansphere Phos-TiO MP Kit

- Centrifuge Adapter, 24 pcs/pk (Cat. No. 5010-21514) is needed to use the Titansphere Phos-TiO MP Kit.
- This centrifuge adapter is reusable.
- Lactic acid is Solution B, and is included in the kit. However, it can be also purchased separately for future uses.

Description	Sorbent Mass/Tip Volume	Qty.	Cat.No.
Titansphere Phos-TiO MP Kit	1 mg/200 µL	24 pcs	5010-21282
	3 mg/200 µL	24 pcs	5010-21283
Centrifuge Adapter	–	24 pcs	5010-21514
Lactic acid for Titansphere Phos-TiO (This is Solution B included in the kit)	15 mL	1/pk	5010-21295

Phos-TiO MP Kit with Desalting Columns

- These special packages includes optimized desalting columns/spin tips to be used with Phos-TiO MP Kit.

Package Contents	Package Cat.No.
Titansphere Phos-TiO MP Kit, 1 mg/200 µL, 24 pcs/pk (Cat.No. 5010-21282)	5010-21272
MonoSpin Large type C18, 30 pcs/pk (Cat.No. 7510-11320)	
GL-Tip SDB, 96 pcs/pk (Cat.No. 7820-11200)	

Package Contents	Package Cat.No.
Titansphere Phos-TiO MP Kit, 3 mg/200 µL, 24 pcs/pk (Cat.No. 5010-21283)	5010-21273
MonoSpin Large type C18, 30 pcs/pk (Cat.No. 7510-11320)	
GL-Tip SDB, 96 pcs/pk (Cat.No. 7820-11200)	

Titansphere Phos-TiO Spin Tips

- Spin tips are also available separately.

Description	Sorbent Mass/Tip Volume	Qty.	Cat.No.
Titansphere Phos-TiO Spin Tips	1 mg/200 µL	24 pcs	5010-21316
		96 pcs	5010-21317
	3 mg/200 µL	24 pcs	5010-21307
		96 pcs	5010-21308

Titansphere Phos-TiO Kit

Enrichment of Phosphopeptide Using Spin Tips

This is the previous phosphopeptide enrichment kit that GL Sciences had introduced to the market and this kit became the most popular worldwide. The titanium dioxide particles contained in the spin tips (available in 1 mg/10 µL and 3 mg/200 µL sizes) is specially treated to maximize selectivity of phosphorylated species, and the conditioning and washing buffers contain components to displace the few non-phosphorylated compounds which might originally adhere to the media.

Features

- High Recovery of Singly Phosphorylated Peptides.
- All operation is done using an easy-to-use centrifuge.

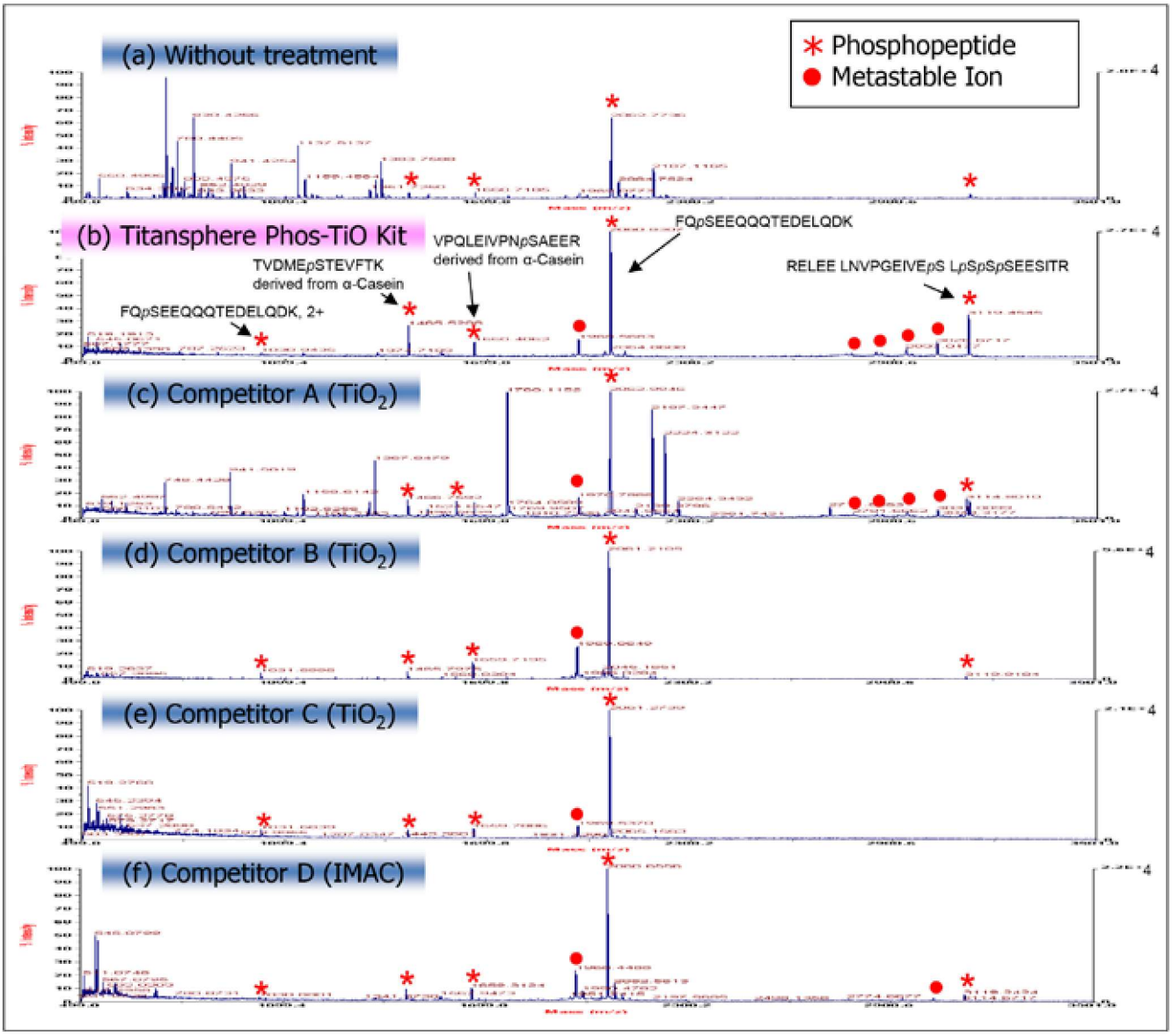
Sample Loading Capacity

Description	Content	
Sample	Tyr (PO_3H_2) - Angiotensin II	
Spin Tip Sorbent Mass/Tip Volume	1 mg/10 µL	3 mg/200 µL
Sample Loading Capacity	1.2 µg	3.5 µg

Titansphere Phos-TiO Kit Contents

Cat.No.	5010-21309	5010-21310	5010-21311	5010-21312
Titansphere Phos-TiO Kit for Export	24 pcs	96 pcs	24 pcs	96 pcs
Titansphere Sorbent Mass/Tip Volume	1 mg/10 µL		3 mg/200 µL	
Spin Tip Quantity	24 pcs (6 x 4 packs)	96 pcs (6 x 16 packs)	24 pcs (6 x 4 packs)	96 pcs (6 x 16 packs)
Waste Fluid Tube Quantity	24 pcs	96 pcs	24 pcs	96 pcs
Recovery Tube Quantity	24 pcs	96 pcs	24 pcs	96 pcs
Solution B (Lactic acid) Quantity	2 mL	6 mL	2 mL	6 mL
Instruction Manual	1/pk	1/pk	1/pk	1/pk

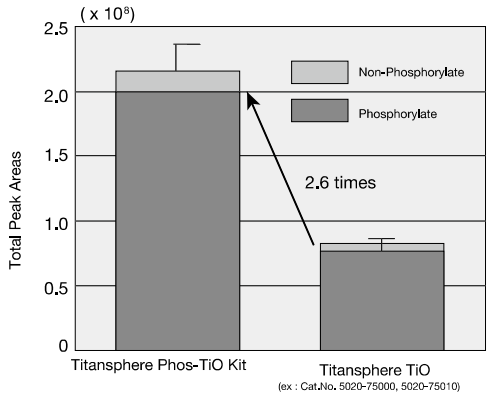
Phos-TiO Kits Outperform 4 Competitive TiO Based Products (MALDI-TOF/MS)



The data above show the purification efficiency of various TiO based products with a 2.5 µg sample of B-casein digest using MALDI-TOF/MS. Compared to the untreated condition (a), phosphopeptides were selectively purified when using Titansphere Phos-TiO Kit. Compared to competitive products (c to e) Titanpshere Phos-TiO Kit showed better selectivity. In general titanium dioxide is said that it has the worse adsorption efficiency of multi-phosphopeptides than IMAC. However, Titansphere Phos-TiO Kit showed higher selectivity, sensitivity and number of individual phosphopeptides isolated for 4 – phosphopeptides than IMAC (f). Metastable ion is a dephosphorylated peak.

Comparison between Previous Version Bulk Material

Optimal TiO beads (Titansphere Phos-TiO Bulk 10 µm, Cat No. 5010-21315) are used for Titansphere Phos-TiO Kit. The existing Titansphere TiO bulk material have been improved for better adsorption capacity of phosphopeptides. Compared to the existing Titansphere bulk material (ex : Cat No. 5020-75000, 5020-75010), Phos-TiO Kit showed 2.6 times more peak area and 1.6 times more identified phosphopeptides.



Sample : HeLa Cell Lysate
Sample Volume : 50 µg
Titansphere TiO Media : 1 mg

Identified Numbers of Phosphopeptides

Product	Phosphorylate	Non-Phosphorylate
Titansphere Phos-TiO Kit	996	185
Titansphere TiO Bulk Media (ex : Cat.No. 5020-75000, 5020-75010)	635	71

Titansphere Phos-TiO Kit

- Centrifuge Adapter, 24 pcs/pk (Cat. No. 5010-21514) is needed once to use the Titansphere Phos-TiO Kit.
- This centrifuge adapter is reusable.
- Lactic acid is Solution B, which is already included in the kit. However, it can be also be purchased separately for future uses.

Description	Sorbent Mass/Tip Volume	Qty.	Cat.No.
Titansphere Phos-TiO Kit	1 mg/10 µL	24 pcs	5010-21309
		96 pcs	5010-21310
Titansphere Phos-TiO Kit	3 mg/200 µL	24 pcs	5010-21311
		96 pcs	5010-21312
Centrifuge Adapter	–	24 pcs	5010-21514
Lactic acid for Titansphere Phos-TiO (This is Solution B included in the kit)	15 mL	1/pk	5010-21295

Titansphere Phos-TiO Spin Tips

- Spin tips are also available separately.

Description	Sorbent Mass/Tip Volume	Qty.	Cat.No.
Titansphere Phos-TiO Spin Tips	1 mg/10 µL	24 pcs	5010-21302
		96 pcs	5010-21303
	3 mg/200 µL	24 pcs	5010-21307
		96 pcs	5010-21308

Titansphere Phos-TiO for Large Volume Samples

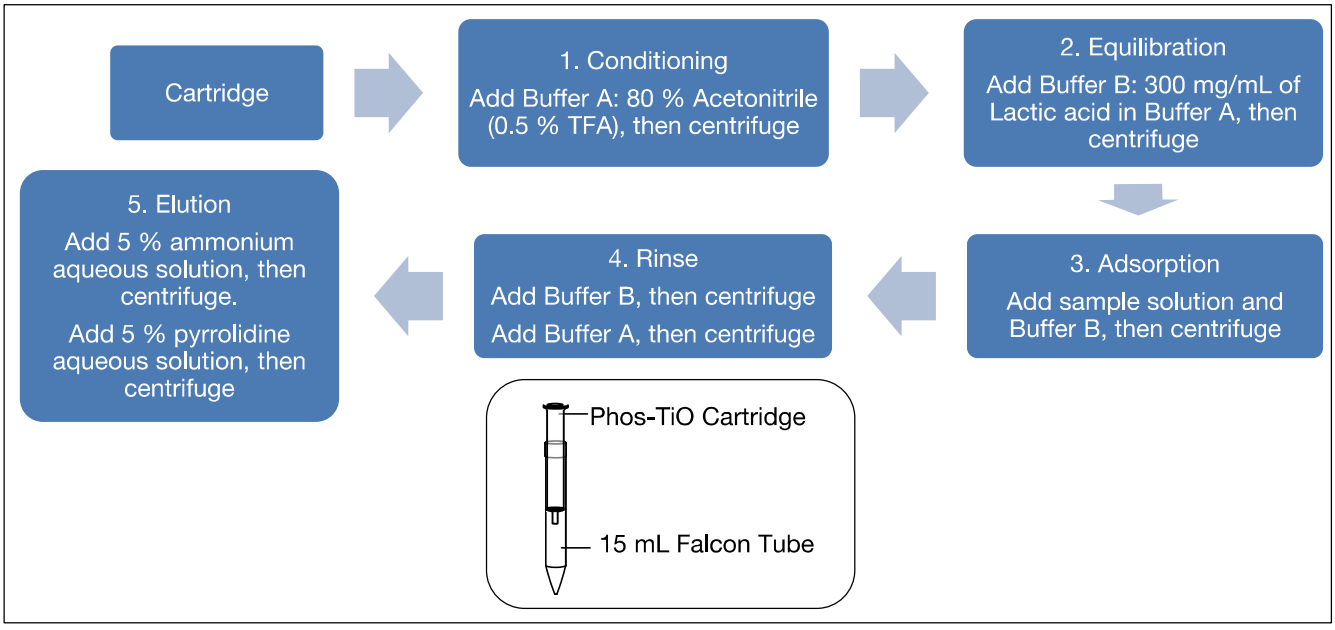
Appropriate for Larger Scale/Volume Purifications

The same specialized bulk media used in our Phos-TiO Kit is available in 50 mg/3 mL and 100 mg/3 mL cartridges as an extension of the Phos-TiO product line.

Sample Loading Capacity

Description	Content	
Sample	Tyr (PO_3H_2) - Angiotensin II	
Spin Tip Sorbent Mass/Tip Volume	50 mg/3 mL	100 mg/3 mL
Sample Loading Capacity	50 µg	100 µg

Typical Enrichment Protocol using Titansphere Phos-TiO for Large Volume Samples



Phos-TiO for Large Volume Samples Cartridges are intended for use with a desktop or other centrifuge. While some of the versions of Phos-TiO are resemble pipette tips or SPE cartridges, these products are not intended for use with pipettes or SPE vacuum manifolds; the cartridge internal configuration and particle size of the TiO bulk media requires centrifugal elution of all solutions.

Titansphere Phos-TiO for Large Volume Samples

Description	Sorbent Mass/Tip Volume	Qty.	Cat.No.
Titansphere Phos-TiO for Large Volume Samples	50 mg/3 mL	25 pcs	5010-21290
	100 mg/3 mL	25 pcs	5010-21291

GL-Tip SDB and GL-Tip GC

Desalting Phosphopeptide-Enriched Samples Prior to LC-MS/MS

Phosphopeptides isolated using TiO_2-based media are typically desalted prior to analysis by LC-MS/MS, typically using a C18 (hydrophobic) micropipette tip. GL Sciences' SDB (styrene divinylbenzene) and GC (graphite carbon) centrifuge-operated micropipette GL-Tip retain more hydrophobic and hydrophilic peptides, respectively, than C18-based tips.

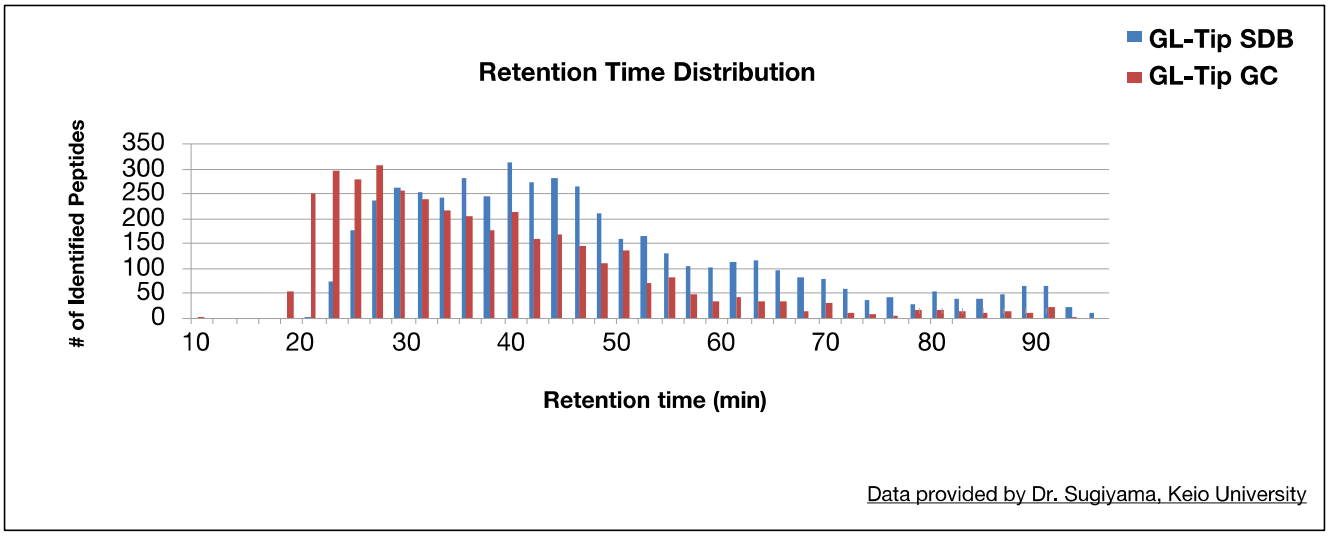
Features

GL-Tip SDB are more hydrophobic than C18 media and retain a wider range of phosphopeptides with high yield, allowing more accurate analysis of phosphopeptides species present in the sample. GL-Tip GC retain many more hydrophilic phosphopeptides than does C18; by using a combination of GL-Tip SDB and GC, almost all peptide samples can be desalted without sample losses due to lack of retention. GL-Tip SDB is designed to be easy-to-use: phosphopeptide-enriched. Phosphopeptide-enriched samples are easily loaded, washed, and eluted using the same centrifuge-based technique used with Phos-TiO spin tips.

Sample Loading Capacity

Description	GL-Tip SDB	GL-Tip GC
Sample	Tyr(PO_3H_2) - Angiotensin II	Gly-Gly-Tyr-Arg
Spin Tip Sorbent Mass/Tip Volume	200 µL	1 mg/200 µL
Sample Loading Capacity	60 µg	30 µg

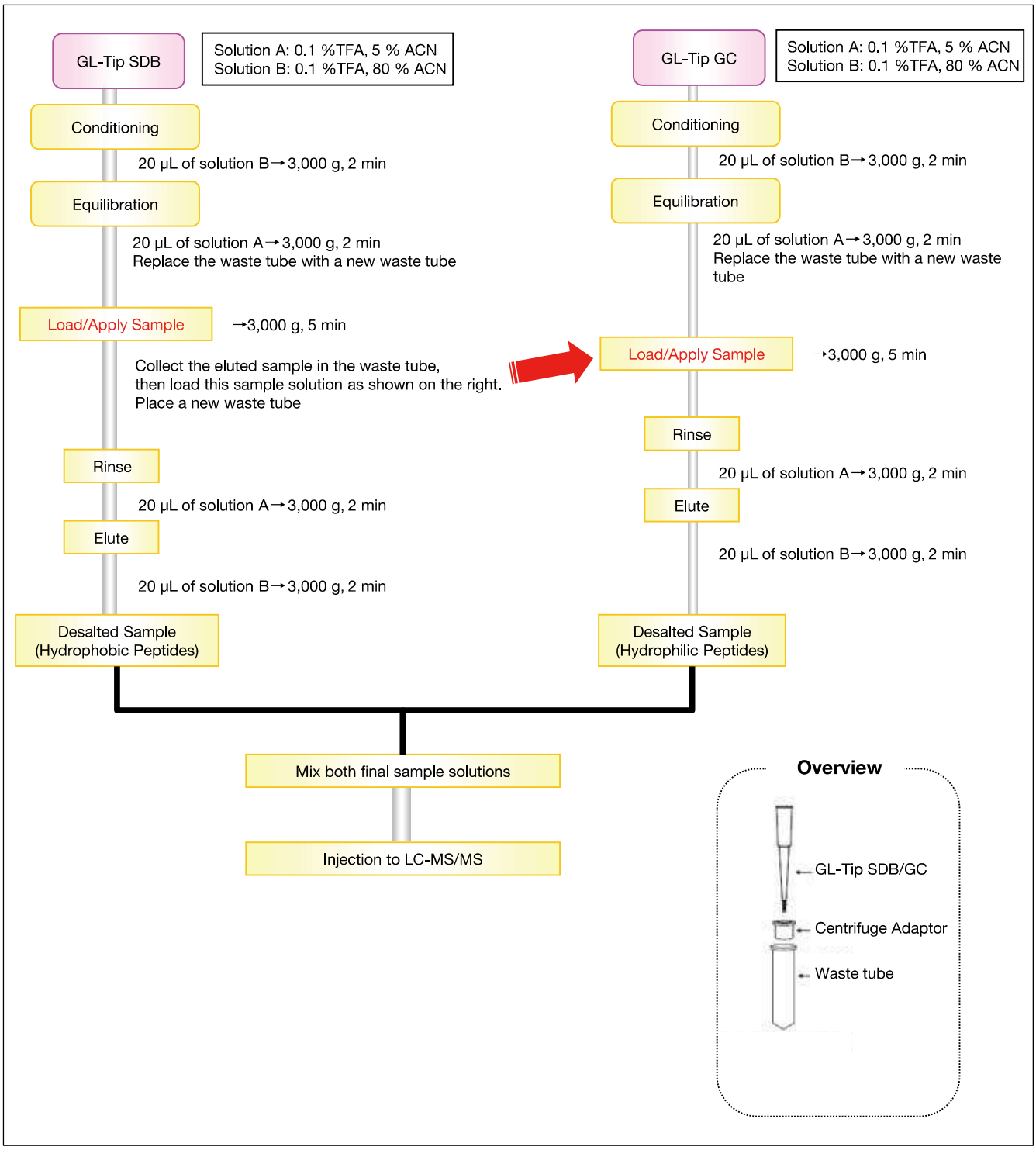
Relative Retention of Peptides Collected using GL-Tip SDB and GC Desalting Tips



Data provided by Dr. Sugiyama, Keio University

As shown above GL-Tip SDB preferentially binds hydrophobic peptides while GC preferentially binds hydrophilic peptides.

Recommended Protocol using GL-Tip SDB and GL-Tip GC



GL-Tip SDB and GL-Tip GC

- Centrifuge Adapter, 24 pcs/pk (Cat. No. 5010-21514) is needed to use the GL-Tip SDB and GL-Tip GC desalting spin tips.
- This centrifuge adapter is reusable.

Description	Tip Volume	Qty.	Cat.No.
GL-Tip SDB	200 µL	96 pcs	7820-11200
GL-Tip GC	200 µL	96 pcs	7820-11201
Centrifuge Adapter	–	24 pcs	5010-21514

GL-Tip SCX and GL-Tip SDB-SCX

Spin Tips for Peptide Fractionation

GL-Tip SCX is packed with strong cation polymer (SCX) and GL-Tip SDB-SCX are packed with styrene divinylbenzene polymer (SDB) and strong cation polymer (SCX). GL-Tip SDB-SCX is packed in a two layer format consisting of a SDB and SCX media. Undesalted peptide samples can be used in GL-Tip SDB-SCX as the first SDB layer makes the first desalination, while the SCX layer desalts the remaining not desalted peptides.

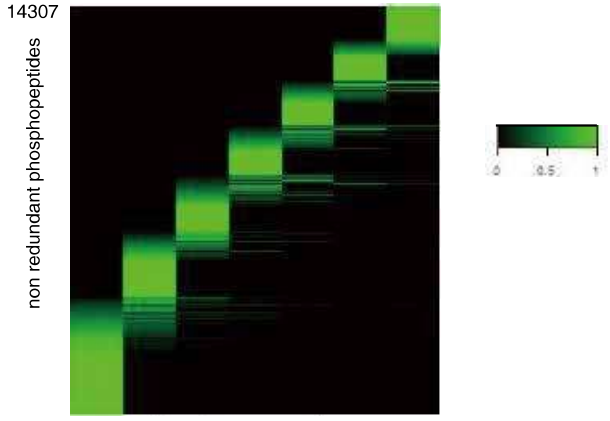
Sample Loading Capacity

Description	GL-Tip SCX	GL-Tip SDB-SCX
Sample	Angiotensin II	Angiotensin II
Tip Volume	200 µL	200 µL
Sample Loading Capacity	60 µg	60 µg

Comparison of Traditional Gradient Elution vs TFA Gradient Elution

Comparison of Efficiency between Traditional Gradient Elution vs TFA Gradient Elution using DLD-1 Human Large Intestinal Cancer Cell derived Phosphopeptides

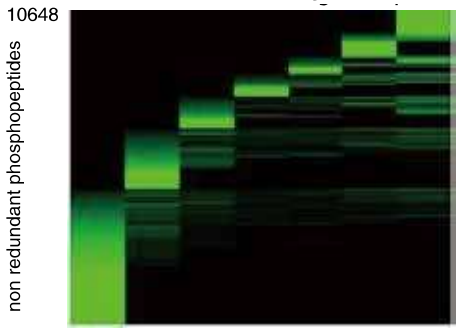
< NEW TFA Gradient Elution using GL-Tip SDB-SCX >



Fraction	1	2	3	4	5	6	7
TFA (%)	0	1	2	3	3	4	0
Ammonium acetate (mM)	0	0	0	0	100	500	500

Concentration in elution buffer

< Traditional Gradient Elution using GL-Tip SDB-SCX >



Fraction	1	2	3	4	5	6	7
Ammonium acetate (mM)	20	50	75	125	200	500	0
NH_4OH (%)	0	0	0	0	0	0	0.1

Concentration in elution buffer

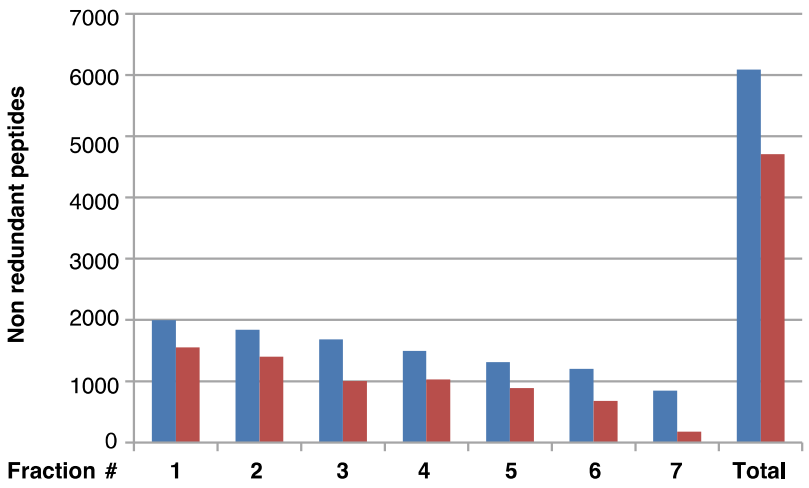
As proven above, the newly developed TFA gradient elution identified 14307 peptides promising higher efficiency than the traditional gradient elution method.

(Data provided from the National Institute of Biomedical Innovation, Dr. Jun Adachi)

A gradient elution using cation SCX media is commonly used in shotgun proteomics to fractionate peptide samples from complex samples such as cell or tissue extracts. The biggest challenge arises when identifying the same peptide from one fractionated peptide sample to another because this may affect efficiency in a bad fashion. efficiency. The newly developed TFA gradient elution method (patent applied) identifies more peptides without decreasing operation efficiency.

Comparison of Number of Quantified Peptides

The figure below shows a comparison between the analysis results obtained respectively by GL-Tip SCX and another brand tip column which we will call below Brand G. GL-Tip SCX recovered more peptides. The usage of the newly developed TFA gradient elution method prevents from identifying the same peptide from one fractionated sample peptide to another resulting in higher efficiency.



Sample Procedure :
Trypsin digestion of HeLa cell lysate 25 µg each were fractionated and 40 % of the recovered sample were identified via LTQ-Orbitrap XL, 45 minutes gradient using Maxquant 1.5.1.2, uniprot human database.

Number of Identified Non Redundant Peptides

Fractions	1	2	3	4	5	6	7	total
GL-Tip SCX	1996	1839	1684	1491	1311	1196	847	6085
Brand G	1552	1397	1004	1032	890	676	179	4704

(Data provided from the National Institute of Biomedical Innovation, Dr. Jun Adachi)

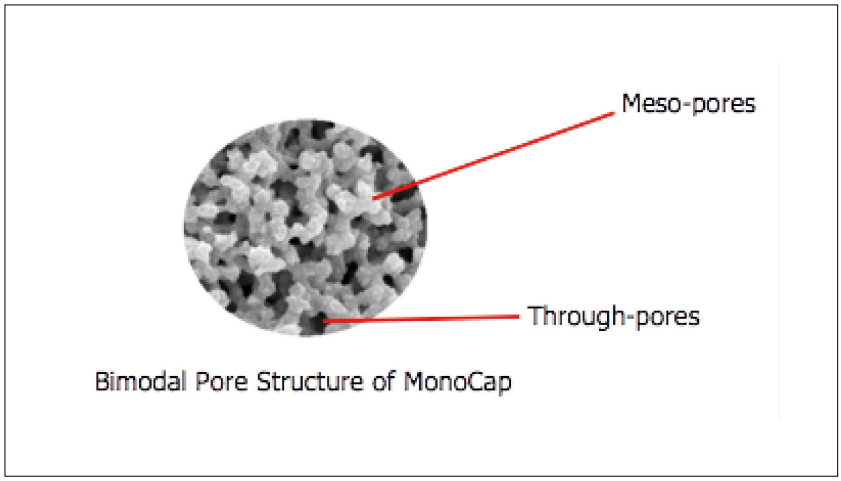
GL-Tip SCX and GL-Tip SDB-SCX

- Centrifuge Adapter, 24 pcs / pk (Cat. No. 5010-21514) is needed to use the GL-Tip SCX and GL-Tip SDB-SCX peptide fractionation spin tips.
- This centrifuge adapter is reusable.

Description	Tip Volume	Qty.	Cat.No.
GL-Tip SCX	200 µL	96 pcs	7510-11203
GL-Tip SDB-SCX	200 µL	96 pcs	7510-11202
Centrifuge Adapter	–	24 pcs	5010-21514
Centrifuge Adapter for 96-Well Plate	–	1 pc	5010-21341
Centrifuge Adapter for 96-Well Plate	–	2 pcs	5010-21343

MonoCap HighResolution 2000 Series

Optimized for Identification of Peptides/Proteins for Proteome Research



Bimodal Pore Structure of MonoCap

MonoCap HighResolution 2000 is a 2 meter length monolithic silica capillary column which is designed for identifying extremely high number of peptides/proteins for proteome research via LC-MS/MS.

GL Sciences' MonoCap capillary columns are created via sol-gel processing and octadecylsilylated. The monolithic structure of MonoCap has a very uniform three dimensional structure that shows excellent reproducibility from batch-to-batch.
Due to the solid structure of GL Sciences' monolithic silica, no frits or filters at the ends of the column. In this way band broadening caused by dead volume is avoided.
The high porosity of our monolithic silica allows for high flow rates to be used without loss of resolution or of high operating pressure. An optimized balance of through-pores and meso-pores provides the critically important combination of efficiency, separation speed, large volume sample-loading, and small volume sample-recovery.

MonoCap HighResolution provides extremely high efficiency, delivering over 200,000 plates for a 2,000 mm length column. The MonoCap HighResolution Ultra type delivers over 300,000 plates.

Physical Properties

Product Description	Bonded Phase	Meso-pore	End-capping	Max. Operating Pressure
MonoCap C18 HighResolution 2000	Octadecyl Groups	15 nm	Yes	35 MPa
MonoCap C18 HighResolution Ultra 2000	Octadecyl Groups	11 nm	Yes	35 MPa
MonoCap HILIC-UP HighResolution 2000	Ureidopropyl Groups	12 nm	None	35 MPa

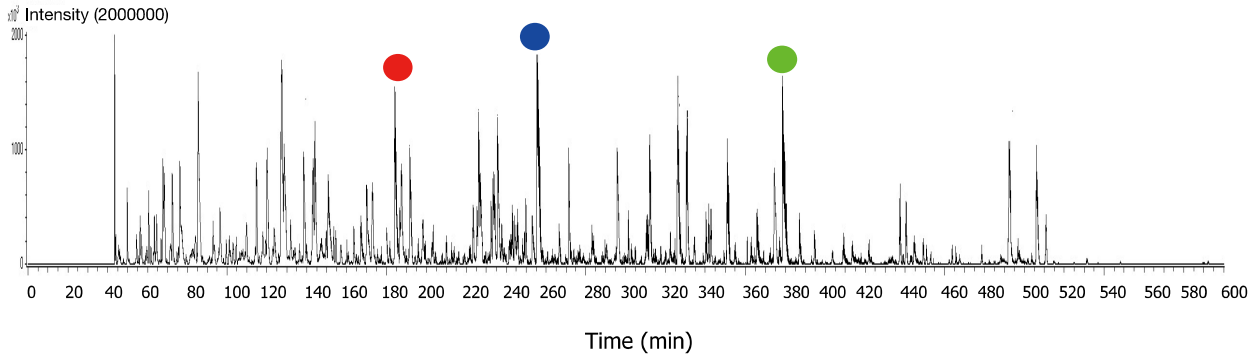
Discover New Peptides/Proteins

As proven below, MonoCap C18 HighResolution 2000 mm length column identifies more peptides/proteins than traditional particle packed capillary HPLC columns.

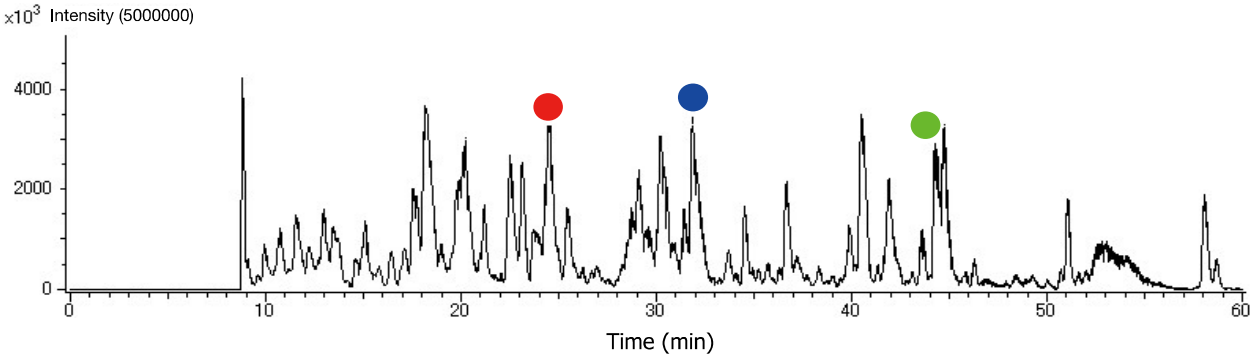
MS : LTQ-Orbitrap XL (Mascot Search)
Sample : THP-1 Cell Lysate Tryptic Digest, 5 µg

Column Name	Number of Identified Proteins in average	Analysis Time
MonoCap C18 HighResolution 2000 0.1 mm I.D. x 2000 mm	2,087 (2013, 2116, 2131)	10 Hours
Particle packed column 0.1 mm I.D. x 150 mm	680 (685, 679, 675)	2 Hours

[1] MonoCap C18 HighResolution 2000 (2000 mm x 0.1 mm I.D.)



[2] Particle Packed column (3 µm, 150 mm x 0.075 mm I.D.)



Conditions

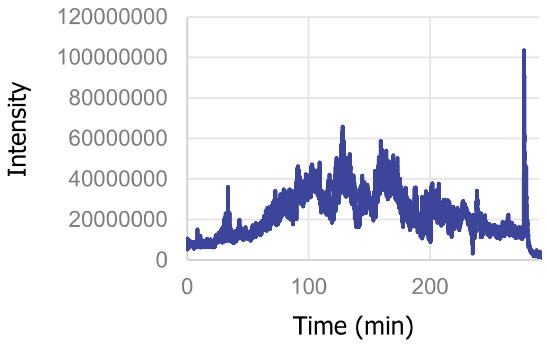
System : GLS Capillary HPLC system
Column : [1] MonoCap C18 High Resolution 2000 (2000 mm x 0.1 mm I.D)
: [2] Particle packed column (3 µm, 150 mm x 0.075 mm I.D.)
Trap column : MonoCap C18 Trap Column (50 mm x 0.075 mm I.D.)
Eluent : A) 0.1 %HCOOH in CH_3CN
B) 0.1 %HCOOH in H_2O
[1] A/B = 10/90 - 600 min - 45/55
[2] A/B = 10/90 - 180 min - 45/55

Flow Rate : [1] 0.5 µL/min
[2] 0.3 µL/min
Injection Vol. : 5 µL
Detection : MS (TIC m/z 500-1500)
Sample : Tryptic digest of proteins

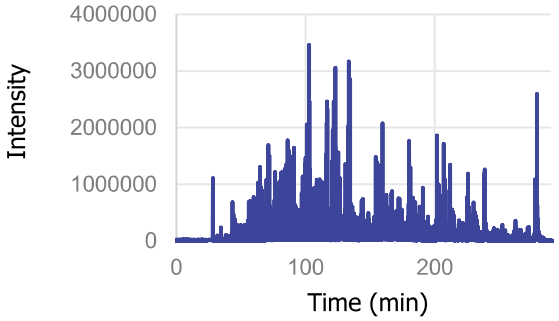
● ● ● are analytes having the same molecular weight

For Identifying Highly Hydrophilic, Hydrophobic Peptides/Proteins

MonoCap HILIC-UP is an important addition to the MonoCap C18 HighResolution 2000 column series.
MonoCap HILIC-UP can retain highly hydrophilic peptides/proteins which may lead to discovering new peptides/proteins which a C18 phase cannot identify.
In HILIC, the higher the organic concentration, the greater the retention of more polar analytes. One of the biggest benefit of HILIC mode is, a high organic solvent concentration of the mobile phase will lead to a high sensitivity LC-MS/MS analysis.



Total Ion Chromatogram



Base Peak Chromatogram

Remarks

Results of MonoCap C18 HighResolution 2000

Number of Identified Peptides : 8,358
Number of Identified Proteins : 1,992
Gradient Program : 4 hrs

Results of MonoCap HILIC-UP HighResolution 2000

Number of Identified Peptides : 7,194 (14,736 PSM*)
Number of Identified Proteins : 2,201
*: Peptide Spectrum Match

Conditions

Column : MonoCap HILIC-UP High Resolution 2000
Eluent : A) CH_3CN : H_2O=10/90 (0.5 % CH_3COOH)
B) CH_3CN : H_2O=95/5 (0.5 % CH_3COOH)
A/B=0/100 - (240 min) - 20/80 - (10 min) - 100/0 - (10 min) - 100/0
Flow Rate : 0.5 µL
Injection Vol. : 1 µL (1 mg/mL)
Detection : TIC MS (m/z 300-1500)
Sample : Tryptic Digest of Hela Cell Lysate, 5 µg

Reference

Hydrophilic Interaction Chromatography Using a Meter-Scale Monolithic silica capillary Column for Proteomics LC-MS,
K Horie et al. *Anal. Chem.* 2014, 86, 3817-3824

References

1. M.H.M. van de Meent et al.
   Improvement of the liquid-chromatographic analysis of protein tryptic digests by the use of long-capillary monolithic columns with UV and MS detection, Anal Bioanal Chem, 2007,388, 195-200.

2. Mio Iwasaki et al.
   One-Dimensional Capillary Liquid Chromatographic Separation Coupled with Tandem Mass Spectorometry Unveils the Escherichia coli Proteome on a Microarray Scale, Anal. Chem. 2010, 82, 2616-2620.

3. Mio Iwasaki et al.
   Human Proteome analysis by using reversed phase monolithic silica capillary columns with enhanced sensitivity, J Chromatogr A 2012, 1228, 292-297.

4. Ryota Yamana ea al.
   Rapid and deep profiling of human induced pluripotent stem cell proteome by one-shot NanoLC-MS/MS analysis with meter-scale monolithic silica columns, J Proteome Res. 2013, 12, 214-21.

5. Mari Ogawa-Ohnishi et al.
   Identification of three hydroxyproline O-arabinosyltransferases in Arabidopsis thaliana, Nature Chem. Biol. 2013, 9, 726-730.

6. Satoru Okamoto et al.
   Root-derived CLE glycopeptides control nodulation by direct binding to HAR1 receptor kinase, Nature Commun. 2013,4, 2191.

7. Kanta Horie et al.
   Hydrophilic interaction chromatography using a meter-scale monolithic silica capillary column for proteomics LC-MS, Anal. Chem. 2014, 86, 3817-3824.

Proteomics

MonoCap C18 HighResolution Ultra 2000

- End-fittings are not included.
- The column connection kit is available separately to ensure proper connections.
- Please refer to the below ordering information.

Description	I.D.	Length	Qty.	Cat.No.
MonoCap C18 HighResolution Ultra 2000	0.1 mm	2000 mm	1 pc	5020-10018

MonoCap C18 HighResolution 2000

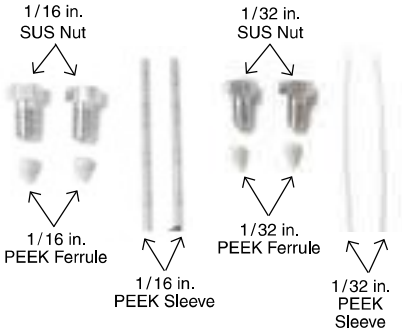
- End-fittings are not included.
- The column connection kit is available separately to ensure proper connections.
- Please refer to the below ordering information.

Description	I.D.	Length	Qty.	Cat.No.
MonoCap C18 HighResolution 2000	0.1 mm	2000 mm	1 pc	5020-10015

MonoCap HILIC-UP HighResolution 2000

- End-fittings are not included.
- The column connection kit is available separately to ensure proper connections.
- Please refer to the below ordering information.

Description	I.D.	Length	Qty.	Cat.No.
MonoCap HILIC-UP HighResolution 2000	0.1 mm	2000 mm	1 pc	5020-10019



Connection Kit for MonoCap HighResolution 2000

- Dedicated connection kit for MonoCap C18 High Resolution 2000.
- Use this connection kit when connecting the column directly to the system.

Description	Qty.	Cat.No.
1/16 in. PEEK Ferrule, SUS Nut, Sleeve, 2 pcs each. 1/32 in. PEEK Ferrule, SUS Nut, Sleeve, 2 pcs each.	1 pc	5020-10017

Zero Dead Volume Union

- The union is used to connect the system tubing to the column to achieve zero dead volume.

Description	Orifice Size	Qty.	Cat.No.
U-435	0.25 mm	1 pc	6010-72352
U-411	178 µm	1 pc	6010-72351

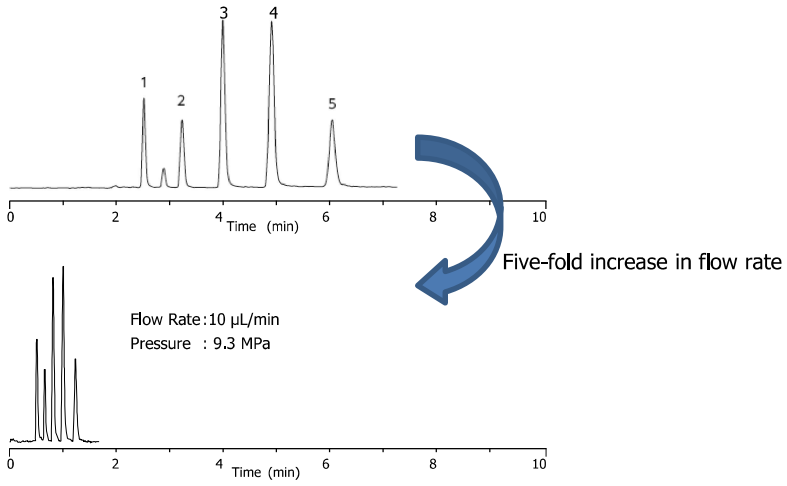
MonoCap C18 Fast-Flow

Physical Properties

Product Description	Bonded Phase	Meso-pore	End-capping	Max. Operating Pressure
MonoCap C18 Fast-flow	Octadecyl Groups	15 nm	Yes	22 MPa

Workable at a broad range of linear velocity from 0.5 to 5 mm/s without sacrificing efficiency and separation at high speed. The number of theoretical plates produced by MonoCap C18 Fast-Flow is nearly equivalent to a totally porous particle type capillary column packed with a 5 µm packing material. Columns are protected by either metal or PEEK hardware.

Workable at High Flow Rates without Sacrificing Efficiency



Conditions

Column : MonoCap C18 for Fast-flow (150 x 0.2 mm I.D.)
Eluent : A) CH_3CN B) H_2O A/B=50/50, v/v
Col.Temp. : Ambient
Detection : UV 210 nm (MU701, Cell Volume 18 nL)
Injection Vol. : 0.5 µL
Sample : 1. Thiourea
2. Acetophenone
3. Benzene
4. Toluene
5. Naphthalene

Proteomics

MonoCap C18 Nano-flow

Physical Properties

Product Description	Bonded Phase	Meso-pore	End-capping	Max. Operating Pressure
MonoCap C18 Nano-flow	Octadecyl Groups	11 nm	Yes	22 MPa

MonoCap C18 Nano-flow delivers higher number of theoretical plates compared to a totally porous particle type capillary column packed with a 3 µm packing material. It can be operated at a wide range of flow rate with low back pressure and achieve very high sensitive results in Nano-LC-ESI/MS applications. Columns are protected by either metal or PEEK hardware.

MonoCap C18 WideBore

Physical Properties

Product Description	Bonded Phase	Meso-pore	End-capping	Max. Operating Pressure
MonoCap C18 WideBore	Octadecyl Groups	11 nm	Yes	22 MPa

The MonoCap C18 Fast-flow is also available in 0.5 mm I.D. size, which can be used at a wide range of flow rate from 6 to 100 µL/min without sacrificing efficiency. The number of theoretical plates delivered by MonoCap C18 WideBore is nearly equivalent to a totally porous particle type capillary column packed with a 5 µm packing material. Columns are protected by a metal hardware.

MonoCap C18 Trap Column

Physical Properties

Product Description	Bonded Phase	Meso-pore	End-capping	Max. Operating Pressure
MonoCap C18 Trap Column	Octadecyl Groups	11 nm	Yes	20 MPa

MonoCap C18 Trap columns have a relatively big throughpore and can be used at high flow rates such as 10 µL/min. This benefit makes MonoCap C18 Trap columns appropriate for on-line preconcentration or desalting of protein and peptide samples prior to HPLC separation with mass spectrometry detection. End-fittings are 1/16" (10-32 UNF). 1/32" end-fittings are also available upon request.

MonoCap Amide

Physical Properties

Product Description	Bonded Phase	Meso-pore	End-capping	Max. Operating Pressure
MonoCap Amide	Carbamoyl Groups	15 nm	None	22 MPa

Amide groups are chemically bonded to the monolithic silica and makes it suitable for the analysis of sugars via HILIC mode. As the back pressure is significantly low, a 500 mm length MonoCap Amide column delivers over 40,000 plates offering high efficiency. Generally, HILIC mode uses acetonitrile at a concentration between 65-95 % in an aqueous buffer such as ammonium acetate or ammonium formate, which have high solubility in organic solvents. Columns are protected by either metal or PEEK hardware.

MonoCap SCX

Physical Properties

Product Description	Bonded Phase	Meso-pore	End-capping	Max. Operating Pressure
MonoCap SCX	Benzenesulfonyl Groups	11 nm	None	20 MPa

MonoCap SCX is bonded with benzene sulfonic acid groups (strong cation exchange) and is suitable for 2D LC applications for the separation of biomolecules such as peptides and proteins.

MonoCap C18 Fast-Flow

- For end-fittings information, please refer to P82
- All 50 mm length PEEK columns does not come with a hardware and will be supplied with 3 pcs of columns only.

Description	I.D.	Length	Hardware	Qty.	Cat.No.
MonoCap C18 Fast-Flow	0.05 mm	50 mm	Metal	1 pc	5020-10102
		150 mm		1 pc	5020-10101
		250 mm		1 pc	5020-10100
		50 mm	PEEK	3 pcs	5020-10002
		150 mm		1 pc	5020-10001
		250 mm		1 pc	5020-10000
	0.075 mm	50 mm	Metal	1 pc	5020-10211
		150 mm		1 pc	5020-10212
		250 mm		1 pc	5020-10213
		50 mm	PEEK	3 pcs	5020-10221
		150 mm		1 pc	5020-10222
		250 mm		1 pc	5020-10223
	0.1 mm	50 mm	Metal	1 pc	5020-10112
		150 mm		1 pc	5020-10111
		250 mm		1 pc	5020-10110
		50 mm	PEEK	3 pcs	5020-10012
		150 mm		1 pc	5020-10011
		250 mm		1 pc	5020-10010
	0.2 mm	50 mm	Metal	1 pc	5020-10122
		100 mm		1 pc	5020-10124
		150 mm		1 pc	5020-10121
		250 mm		1 pc	5020-10120
		50 mm	PEEK	3 pcs	5020-10022
		150 mm		1 pc	5020-10021
		250 mm		1 pc	5020-10020

MonoCap C18 Nano-Flow

- For end-fittings information, please refer to P82
- All 50 mm length PEEK columns does not come with a hardware and will be supplied with 3 pcs of columns only.

Description	I.D.	Length	Hardware	Qty.	Cat.No.
MonoCap C18 Nano-Flow	0.05 mm	50 mm	Metal	1 pc	5020-10143
		150 mm		1 pc	5020-10141
		50 mm	PEEK	3 pcs	5020-10043
		150 mm		1 pc	5020-10041
	0.075 mm	50 mm	Metal	1 pc	5020-10231
		150 mm		1 pc	5020-10232
		50 mm	PEEK	3 pcs	5020-10241
		150 mm		1 pc	5020-10242
	0.1 mm	50 mm	Metal	1 pc	5020-10153
		150 mm		1 pc	5020-10151
		50 mm	PEEK	3 pcs	5020-10053
		150 mm		1 pc	5020-10051
	0.2 mm	50 mm	Metal	1 pc	5020-10163
		150 mm		1 pc	5020-10161
		50 mm	PEEK	3 pcs	5020-10063
		150 mm		1 pc	5020-10061

MonoCap C18 WideBore

- For end-fittings information, please refer to P84

Description	I.D.	Length	Hardware	Qty.	Cat.No.
MonoCap C18 WideBore	0.5 mm	50 mm	Metal only	1 pc	5020-10202
		150 mm		1 pc	5020-10201
		250 mm		1 pc	5020-10200

MonoCap C18 Trap Column

・For end-fittings information, please refer to P81

Description	I.D.	Length	Hardware	Qty.	Cat.No.
MonoCap C18 Trap Column	0.05 mm	50 mm	With Hardware	1 pc	5020-10026
		100 mm		1 pc	5020-10038
		150 mm		1 pc	Contact Us
		50 mm	Without Hardware	1 pc	5020-10027
		100 mm		1 pc	5020-10039
		150 mm		1 pc	Contact Us
	0.075 mm	50 mm	With Hardware	1 pc	5020-10028
		100 mm		1 pc	5020-10036
		150 mm		1 pc	Contact Us
		50 mm	Without Hardware	1 pc	5020-10029
		100 mm		1 pc	5020-10037
		150 mm		1 pc	Contact Us
	0.2 mm	50 mm	With Hardware	1 pc	5020-10033
		100 mm		1 pc	Contact Us
		150 mm		1 pc	Contact Us
		50 mm	Without Hardware	1 pc	5020-10034
		100 mm		1 pc	Contact Us
		150 mm		1 pc	5020-10031

MonoCap Amide

・For end-fittings information, please refer to P82

Description	I.D.	Length	Hardware	Qty.	Cat.No.
MonoCap Amide	0.075 mm	150 mm	Metal	1 pc	5020-10191
		250 mm		1 pc	5020-10192
		500 mm		1 pc	5020-10193
		150 mm	PEEK	1 pc	5020-10091
		250 mm		1 pc	5020-10092
		500 mm		1 pc	5020-10093
	0.1 mm	150 mm	Metal	1 pc	5020-10181
		250 mm		1 pc	5020-10182
		500 mm		1 pc	5020-10183
		150 mm	PEEK	1 pc	5020-10081
		250 mm		1 pc	5020-10082
		500 mm		1 pc	5020-10083
	0.2 mm	150 mm	Metal	1 pc	5020-10171
		250 mm		1 pc	5020-10172
		500 mm		1 pc	5020-10173
		150 mm	PEEK	1 pc	5020-10071
		250 mm		1 pc	5020-10072
		500 mm		1 pc	5020-10073

MonoCap SCX

・For end-fittings information, please refer to the following information.

Description	I.D.	Length	Hardware	Qty.	Cat.No.
MonoCap SCX	0.2 mm	50 mm	Metal	1 pc	5020-10174
		150 mm		1 pc	5020-10175
		250 mm		1 pc	5020-10176
		500 mm		1 pc	5020-10177
		50 mm	PEEK	1 pc	5020-10074
		150 mm		1 pc	5020-10075
		250 mm		1 pc	5020-10076
		500 mm		1 pc	5020-10077

Connection Kit for MonoCap C18 Trap Column

Description	Cat.No.
MonoCap C18 Trap Column Connection Kit 1/16 in. (Union·Sleeve·Capillary Tubing 2 pcs each, Nut·Ferrule 4 pcs each)	5020-10044
MonoCap C18 Trap Column Connection Kit 1/32 in. (Union·Sleeve·Capillary Tubing 2 pcs each, Nut·Ferrule 4 pcs each)	5020-10045
MonoCap C18 Trap Column Assembly Parts 1/16 in. (Nut·Ferrule 4 pcs each)	5020-10046
MonoCap C18 Trap Column Assembly Parts 1/32 in. (Nut·Ferrule 4 pcs each)	5020-10047

End-fittings of MonoCap Monolithic Capillary HPLC Columns

MonoCap C18 High Resolution 750
MonoCap C18 Fast-flow
MonoCap Nano-flow
MonoCap C18 WideBore
MonoCap Amide
MonoCap SCX

→

1. Metal Hardwares
   End-fittings are Parker Style (UP type).
   Valco 1/32 inch (6-40 UNF) end-fittings are also available upon request, indicate 1/32 inch when ordering.

2. PEEK Hardwares
   1/16 inch male nut, ferrule and PTFE sleeve are included.

MonoCap C18 High Resolution 2000
MonoCap C18 High Resolution Ultra 2000
MonoCap C18 High Resolution 1000
MonoCap C18 High Resolution Ultra 1000
MonoCap HILIC-UP High Resolution

→

End-fittings are not included.
The connection kits shown at page 74 must be purchased separately once.

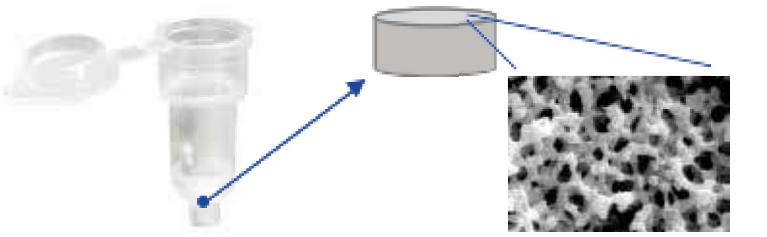
MonoSpin Series

Low-Molecular Compounds Extraction and Purification

The low-pressure, high-flow, and low-liquid-retention properties of GL Sciences' monolith silica technology make it uniquely suited for handling of small samples. MonoSpin SPE centrifugal spin columns have been developed to improve concentration and yields in low-volume sample preparation.

Features

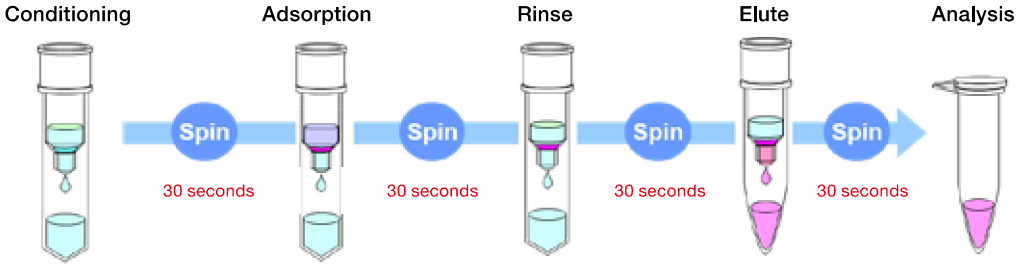
- Easy-to-Operate
- Ideal for Small Sample Volumes
- Wide Variety of Functional Groups
- Rapid Operation Time



MonoSpin　　Silica Monolith Enlarged View

How to Operate

Centrifuge elution allows loss-free and efficient processing of many samples simultaneously, with little or no liquid retained by the separation matrix. And, excellent mass transfer and rapid sample binding on MonoSpin's monolith silica allows extremely rapid sample preparation compared with other methods.



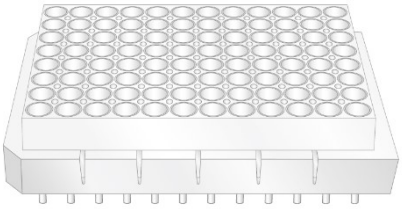
Formats

S Type (Small)

- Disc Size : 4.2 O.D. x 1.5 mm
- Sample Volume : 50 ～ 800 µL
- Elution Volume : 50 ～ 800 µL
- Centrifugation Speed : 2,000 ～ 10,000 x *g*

L Type (Large)

- Disc Size : 9 O.D. x 3 mm
- Sample Volume : 0.5 ～ 8 mL
- Elution Volume : 0.5 ～ 8 mL
- Centrifugation Speed : 1,000 x *g*



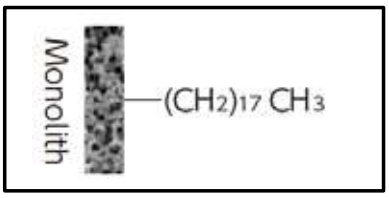
96 Well-Plate

- Dise Size : ～ 800 µL
- Elution Volume : 50 ～ 800 µL
- Centrifugation Speed : 1,000 ～ 5,000 x *g*
  (also can be used in vacuum aspiration)

Sample Preparation for LC/MS

Product Lineup

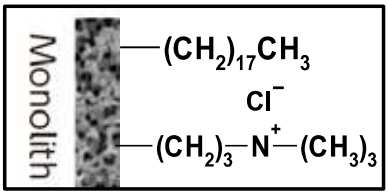
MonoSpin C18/C18 FF



Formats : S L 96

Octadecyl functional group. Optimal for drug extraction in biological samples, and desalting & enrichment of peptide samples C18 FF type employs large through-pore monolith silica for high viscosity samples.

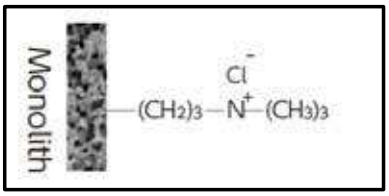
MonoSpin C18-AX



Formats : S 96

Bonded with octadecyl and trimethylaminopropyl, a mix mode type. Delivers great retention for high salt concentrated serum samples. Optimal for the recovery of acidic drugs.

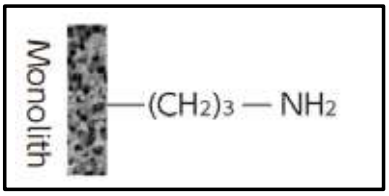
MonoSpin SAX



Formats : S L 96

Bonded with trimethylaminopropyl combining both strong anion exchange & weak hydrophobic interaction. Optimal for the extraction of acidic drugs.

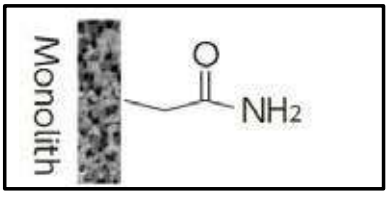
MonoSpin NH2



Formats : S L 96

Bonded with aminopropyl. Optimal for the enrichment of sugar chain and/or hydrophilic compounds by HILIC mode.

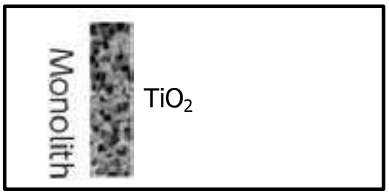
MonoSpin Amide



Formats : S 96

Bonded with amide. Optimal for the extraction of sugar chains and various hydrophilic acidic and basic compounds by HILIC mode.

MonoSpin TiO



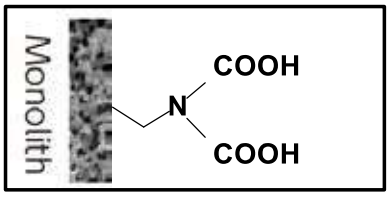
Formats : S

Monolith skeleton coated with titanium dioxide. Excellent for the enrichment of phosphopeptides.

S : Small Type L : Large Type 96 : 96-well plate

Product Lineup

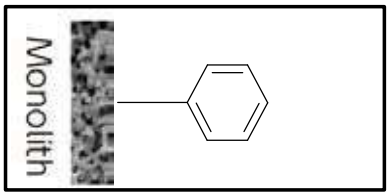
MonoSpin ME



Formats : S L

Bonded with iminodiacetic acid. Optimal for the recovery of trace metals.

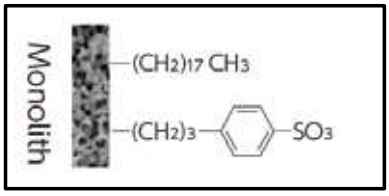
MonoSpin Ph



Formats : S

Phenyl functional group. Optimal for the recovery of hydrophobic drugs in biological samples due to its weak retentivity and different selectivity compared to a C18 phase.

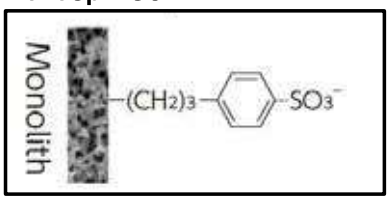
MonoSpin C18-CX



Formats : S 96

Bonded with octadecyl and benzene sulfonic acid combining both ion exchange & hydrophobic interaction. Optimal for dissociated basic drug in biological samples. Delivers higher cleanup efficiency compared to C18 or SCX.

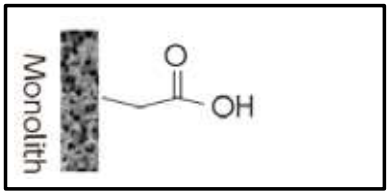
MonoSpin SCX



Formats : S L 96

Bonded with benzenesulfonic acid combining both strong cation exchange & hydrophobic interaction. Optimal for the extraction of basic drugs.

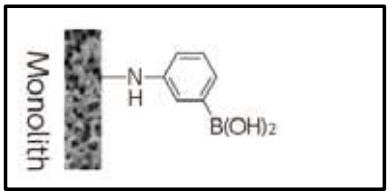
MonoSpin CBA



Formats : S L 96

Bonded with carboxyl acid combining both weak cation exchange. Optimal for the extraction of basic drugs.

MonoSpin PBA



Formats : S 96

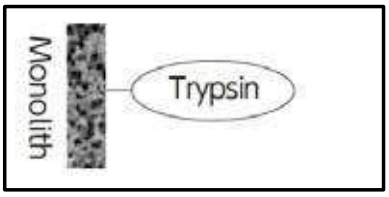
Specific column combined with phenyl boric acid. Excellent for the selective extraction of cis diol compounds, such as catechol amines.

S : Small Type L : Large Type 96 : 96-well plate

Sample Preparation for LC/MS

Product Lineup

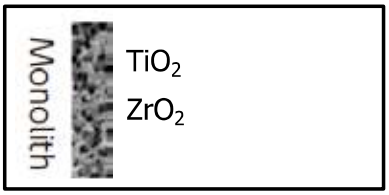
MonoSpin Trypsin



Formats : S

Immobilized trypsin is available for performing rapid and efficient tryptic digests of proteins.

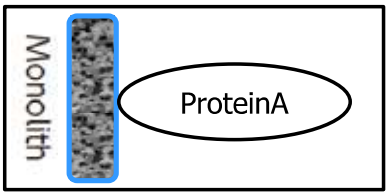
MonoSpin Phospholipid



Formats : S L

Monolith skeleton coated with TiO_2 and ZrO_2. Excellent for the adsorption and removal of phospholipids.

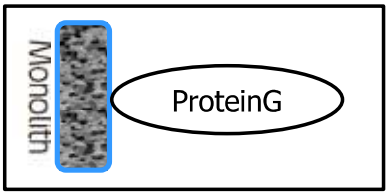
MonoSpin ProA



Formats : S 96

Protein A immobilized affinity spin column for the rapid purification of antibodies.

MonoSpin ProG



Formats : S 96

Protein G immobilized affinity spin column for the rapid purification of antibodies.

S : Small Type L : Large Type 96 : 96-well plate

Physical Properties

Product	Stationary Phases	S Type (Small)		L Type (Large)		Surface Area	Sample Loading Capacity (Small Type)	Filter
		Through-pore	Meso-pore	Through-pore	Meso-pore			
MonoSpin C18	Octadecyl	5 µm	10 nm	10 µm	10 nm	350 m^2/g	100 µg (Amitriptyline)	None
MonoSpin C18 FF	Octadecyl	20 µm	15 nm	–	–	300 m^2/g	50 µg (Amitriptyline)	
MonoSpin Ph	Phenyl	5 µm	10 nm	–	–	350 m^2/g	100 µg (Amitriptyline)	
MonoSpin C18-AX	Octadecyl, Trimethylaminopropyl	5 µm	10 nm	–	–	350 m^2/g	100 µg (Ibuprofen)	
MonoSpin C18-CX	Octadecyl, Benzenesulfonic acid	5 µm	10 nm	–	–	350 m^2/g	100 µg (Amitriptyline)	
MonoSpin SAX	Trimethylaminopropyl	5 µm	10 nm	10 µm	10 nm	350 m^2/g	100 µg (Ibuprofen)	
MonoSpin SCX	Benzenesulfonic acid	5 µm	10 nm	10 µm	10 nm	350 m^2/g	100 µg (Amitriptyline)	
MonoSpin NH2	Aminopropyl	5 µm	10 nm	10 µm	10 nm	350 m^2/g	100 µg (Maltopentaose)	
MonoSpin CBA	Carboxyl	5 µm	10 nm	10 µm	10 nm	350 m^2/g	100 µg (Amitriptyline)	
MonoSpin Amide	Amide	5 µm	10 nm	–	–	350 m^2/g	100 µg (Angiotensin II)	
MonoSpin PBA	Phenyl boric acid	5 µm	10 nm	–	–	350 m^2/g	100 µg (Dopamine)	
MonoSpin TiO	Titanium dioxide	20 µm	15 nm	–	–	200 m^2/g	40 µg (Adenosine monophosphate)	
MonoSpin Trypsin	TPCK treated Trypsin	5 µm	10 nm	–	–	100 m^2/g	–	
MonoSpin ME	Iminodiacetic acid	5 µm	10 nm	10 µm	10 nm	350 m^2/g	25 µg (Cu ion)	
MonoSpin Phospholipid	TiO_2 + ZrO_2	5 µm	10 nm	10 µm	10 nm	350 m^2/g	10 µL (Human serum)	
MonoSpin ProA	Protein A	2 µm	60 nm	–	–	–	400 µg (Human IgG)	
MonoSpin ProG	Protein G	2 µm	60 nm	–	–	–	400 µg (Human IgG)	

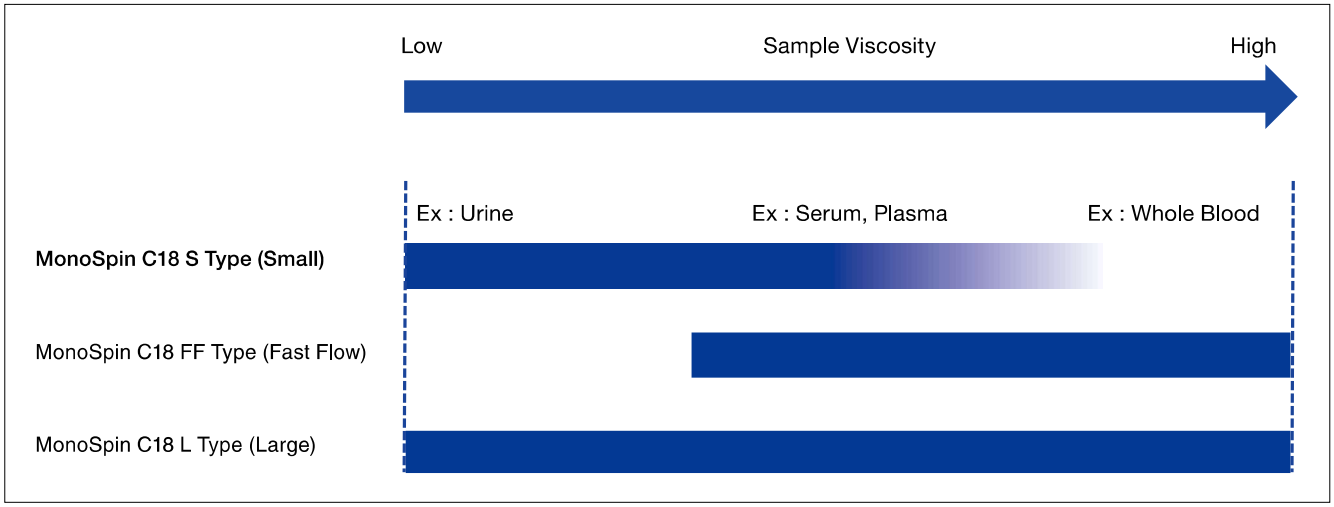
Specifications

Description	MonoSpin S Type*1	MonoSpin FF*2	MonoSpin L Type	MonoSpin 96 Well-Plate
Disc Size	4.2 O.D. x 1.5 mm	4.2 O.D. x 1.5 mm	9 O.D. x 3 mm	4.2 O.D. x 1.5 mm
Sample Volume	50 ~ 800 µL	50 ~ 800 µL	0.5 ~ 8 mL	~ 800 µL
Elution Volume	50 ~ 800 µL	50 ~ 800 µL	0.5 ~ 8 mL	100 ~ 800 µL
Centrifugation Speed	2,000 ~ 10,000 x *g*	1,000 x *g*	1,000 x *g*	1,000 ~ 5,000 x *g*
Sample Loading Capacity	100 µg	50 µg	1 mg	

Sample Preparation for LC/MS

Appropriate for Various Viscosity Samples

MonoSpin series are ideal for the sample preparation of biological samples. MonoSpin C18 Fast Flow (FF) type is excellent for high viscosity biological samples. Select the appropriate MonoSpin column type depending on the viscosity of sample and volume.



MonoSpin Applications

The low-pressure, high-flow, and low-liquid-retention properties of GL Sciences' monolith silica technology make it uniquely suited for handling of small samples. MonoSpin SPE centrifugal spin columns have been developed to improve concentration and yields in low-volume sample preparation with no need for evaporation or reconstitution.

Purification and Enrichment of Trace Samples

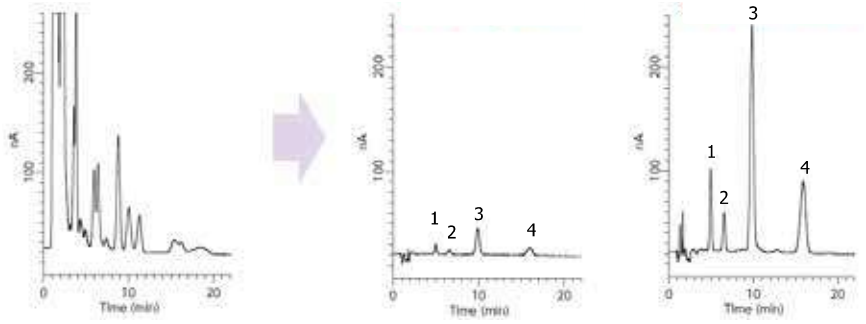
Without MonoSpin PBA

Purification using MonoSpin PBA S Type (Small)

Sample Volume : 500 µL

Elution Volume : 500 µL

Elution Volume : 50 µL
(10-fold concentration)



Conditions

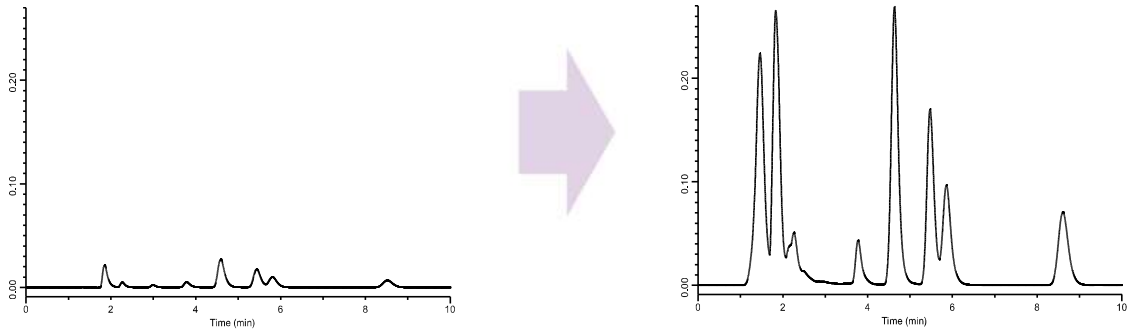
Column : Inertsil ODS-3
(5 µm, 150 mm x 2.1 mm I.D.)
Eluent : 50 mM Phosphate buffer (pH 5.6)
50 mg/L EDTA
600 mg/L IPCC-008
-10 % Methanol
Flow Rate : 0.3 mL/min
Col.Temp. : 35 °C
Injection : 5 µL
Detection : ECD Pulse Mode
Sample : 1. Noradrenaline
2. Adrenaline
3. DHBA
4. Dopamine

Without MonoSpin C18

Purification of Serum using MonoSpin C18 L Type (Large)

Sample Volume : 5000 µL

Elution Volume : 500 µL
(10-fold concentration)



5000 µL serum sample was purified with MonoSpin C18 and enriched by 500 µL.

Conditions

Column : Inertsil ODS-3 (5 µm, 150 x 4.6 mm I.D.)
Eluent : CH_3CN/3 mM KH_2PO_4, 27 mM SDS = 48 = 52
Flow Rate : 1.0 mL/min
Col.Temp : 40 °C
Injection Vol. : 1 µL
Detection : 230 nm

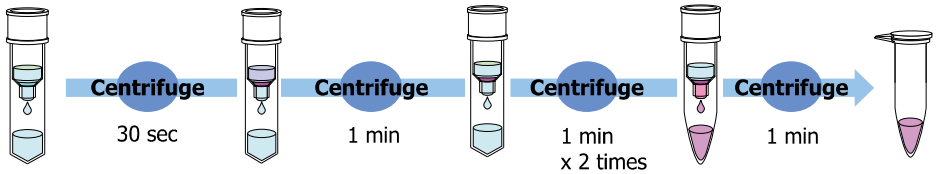
Sample Preparation for LC/MS

Purification of Amphetamine in Urine using MonoSpin C18

Sample Preparation

800 µL sample solution mixed with 400 µL urine and 400 µL buffer solution (pH 13.0).

Centrifugation
Speed : 5,000 x g



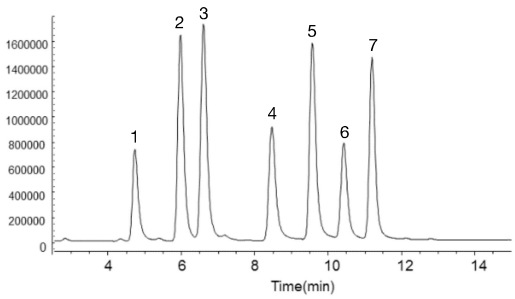
1. Conditioning
Add 100 µL methanol
↓ Centrifuge
Add 300 µL buffer
Solution (pH 13.0)

2. Adsorption
Add 800 µL
sample solution

3. Rinsing
Add 300 µL
buffer solution
(pH 13.0)

4. Elution
Add 100 µL
0.1 % formic
acid-methanol
(1 : 1, v/v)

Purified Sample



※ Data provided from Hiroshima University, Dr. Namera

Conditions

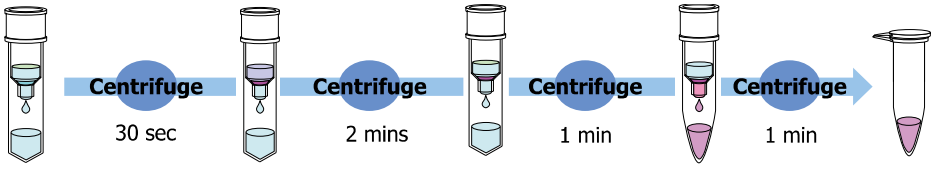
Column : InertSustainSwift C18 (3 µm, 150 x 2.1 mm I.D.)
Eluent : A) 10 mM Ammonium acetate-Formic acid (pH 3.3)
B) CH_3OH
A/B = 90/10 - 2 min - 90/10 - 13 min - 70/30, v/v
Flow Rate : 0.3 mL/min
Col. Temp. : 40 °C
Detection : LC/MS
Sample : 1. Norephedrine
2. Ephedrine
3. Methylephedrine
4. Amphetamine
5. Methamphetamine
6. 3, 4-methylenedioxyamphetamine
7. 3, 4-methylenedioxymethamphetamine

Recovery of Drugs in Serum using MonoSpin C18

Sample Preparation

600 µL sample solution mixed with 200 µL serum and 400 µL 10 mM potassium phosphate buffer solution (pH 7.0).

Centrifugation
Speed : 2,300 x g



1. Conditioning
Add 300 µL methanol
↓ Centrifuge
Add 300 µL 10 mM
potassium
phosphate (pH 7.0)

2. Adsorption
Add 600 µL
sample solution

3. Rinsing
Add 300 µL
water

4. Elution
Add 200 µL
acetonitrile

Purified Sample

Reproducibility study for drugs in serum sample for three days using MonoSpin C18 (n=10)
MonoSpin demonstrated high reproducibility for purification of drugs.

Sample	Con.	Rec.	RSD
Desipramine	5 ng/mL	91.2 %	4.8 %
	10 ng/mL	86.1 %	3.3 %
	50 ng/mL	85.2 %	5.9 %
	250 ng/mL	88.4 %	6.5 %
Imipramine	5 ng/mL	96.3 %	9.5 %
	10 ng/mL	95.8 %	1.5 %
	50 ng/mL	94.5 %	0.9 %
	250 ng/mL	95.9 %	0.9 %
Fluvoxamine	5 ng/mL	96.8 %	11.6 %
	10 ng/mL	87.1 %	5.0 %
	50 ng/mL	86.8 %	8.1 %
	250 ng/mL	87.5 %	9.7 %

Sample	Con.	Rec.	RSD
Paroxetine	5 ng/mL	83.7 %	3.9 %
	10 ng/mL	84.1 %	7.8 %
	50 ng/mL	83.9 %	8.2 %
	250 ng/mL	86.7 %	7.5 %
Maprotiline	5 ng/mL	85.7 %	8.1 %
	10 ng/mL	84.7 %	3.2 %
	50 ng/mL	88.6 %	5.4 %
	250 ng/mL	87.5 %	7.7 %
Duloxetine	5 ng/mL	106.3 %	9.9 %
	10 ng/mL	104.8 %	6.7 %
	50 ng/mL	99.8 %	8.7 %
	250 ng/mL	99.8 %	6.0 %

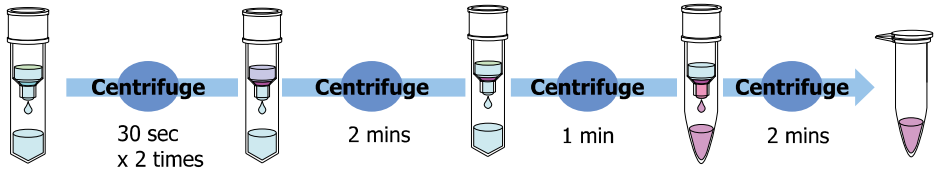
Sample	Con.	Rec.	RSD
Amitriptyline	5 ng/mL	83.7 %	7.0 %
	10 ng/mL	81.8 %	2.8 %
	50 ng/mL	83.8 %	3.0 %
	250 ng/mL	88.4 %	2.7 %
Sulpiride	5 ng/mL	97.9 %	9.0 %
	10 ng/mL	95.5 %	8.5 %
	50 ng/mL	90.8 %	2.6 %
	250 ng/mL	92.6 %	3.0 %

Desalting of Protein Digests using MonoSpin C18

Sample Preparation

800 µL sample solution : Add TFA to tryptic digest sample and adjust the TFA final concentration to 0.1 %.

Centrifugation
Speed : 2,300 x g



1. Conditioning
Add 200 µL acetonitrile
↓ Centrifuge
Add 200 µL 0.1 % TFA aqueous solution

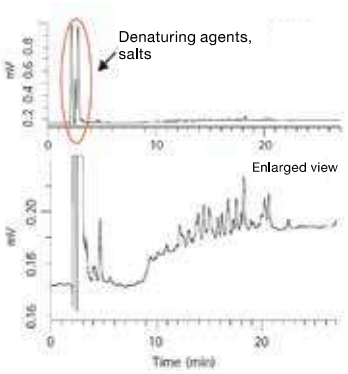
2. Adsorption
Add 800 µL sample solution

3. Rinsing
Add 200 µL 0.1 % TFA aqueous solution

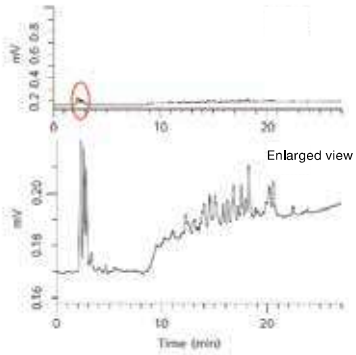
4. Elution
Add 200 µL 60 % acetonitrile

Desalted Sample

Without MonoSpin C18



Desalting using MonoSpin C18



Conditions

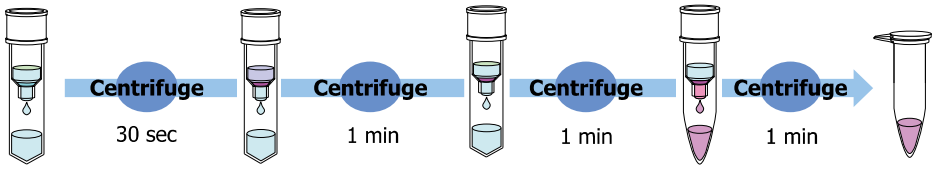
Column	: Inertsil ODS-3 (3 µm, 150 x 2.1 mm I.D.)
Eluent	: A) H_2O (0.1 % TFA) B) Acetonitrile (0.1 % TFA) A/B = 90/10 - 20 min - 50/50
Flow Rate	: UV 210 nm
Col. Temp.	: 0.2 mL/min
Detection	: 40 °C
Sample	: Digested BSA 2 µL

Recovery of Hormone in Serum using MonoSpin C18

Sample Preparation

Add 20 µL of 1 mg/mL of Adrenomedullin to 190 µL of serum. Add 0.1 % TFA to the serum solution and centrifuge at 10,000 x g for 1 min. Take the supernatant.

Centrifugation
Speed : 2,300 x g



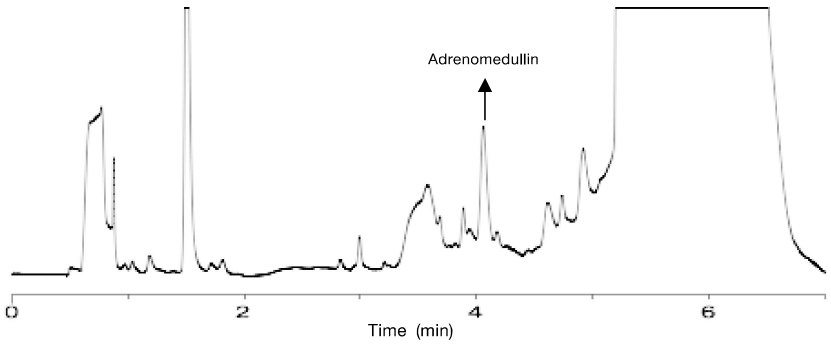
1. Conditioning
Add 200 µL acetonitrile
↓ Centrifuge
Add 200 µL 0.1 % TFA aqueous solution

2. Adsorption
Add 400 µL sample solution

3. Rinsing
Add 200 µL 0.1 % TFA aqueous solution

4. Elution
Add 200 µL 0.1 % TFA in 60 % acetonitrile

Purified Sample



Conditions

Column	: InertSustain C18
	: (2 µm, 50 x 2.1 mm I.D.)
Eluent	: A) 0.1 % TFA in H_2O B) 0.1 % TFA in Acetonitrile A/B = 85/15 – 5 min – 50/50 - 2 min - 50/50
Flow Rate	: 200 µL/min
Col. Temp.	: 40 °C
Detection	: UV 210 nm
Injection Vol.	: 10 µL

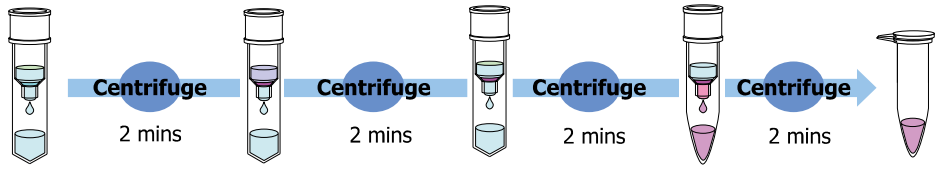
Sample Preparation for LC/MS

Purification of Whole Blood using MonoSpin C18 FF (Fast Flow)

Sample Preparation

Mix 0.3 mL whole blood and 1.2 mL 300 mM phosphate buffer (pH 10). Centrifuge at 12,100 x g for 5 mins and take the supernatant.

Centrifugation Speed : 1,000 x g



1. Conditioning
Add 300 µL methanol
↓ Centrifuge
Add 300 µL 300 mM phosphate buffer (pH 10)

2. Adsorption
Add 1 mL supernatant sample solution

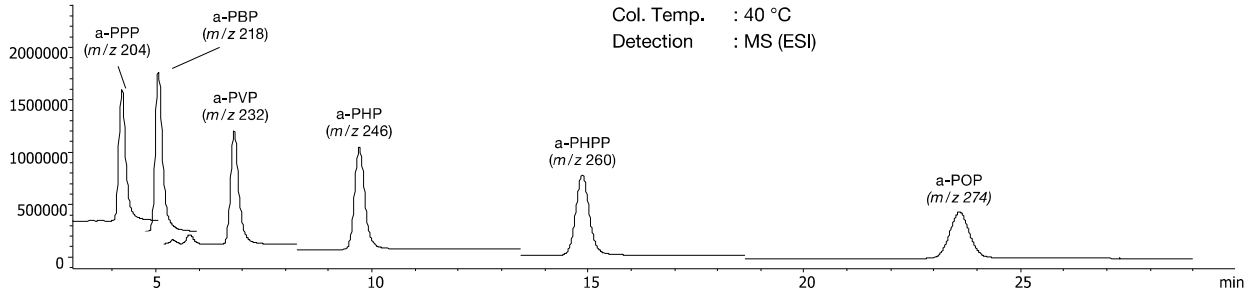
3. Rinsing
Add 300 µL 300 mM phosphate buffer (pH 10)

4. Elution
Add 100 µL methanol

Purified Sample

Conditions

Column : InertSustain Phenyl (3 µm, 150 x 2.1 mm I.D.)
Eluent : acetonitrile-$HCOONH_4$ (10 mM, 0.1 % HCOOH) = 25:75 (v/v)
Flow Rate : 0.2 mL/min
Col. Temp. : 40 °C
Detection : MS (ESI)



Features of MonoSpin C18 FF (Fast Flow)

MonoSpin C18 FF is ideal for high viscosity samples, such as whole blood and complex matrix samples.

Specification

Through-pore	20 µm
Meso-pore	15 nm
Disc Size	4.2 O.D. x 1.5 mm
Sample Volume	50 ~ 800 µL
Elution Volume	50 ~ 800 µL
Centrifugation Speed	Under 1,000 x *g*
Sample Loading Capacity	50 µg (Amitriptyline)

MonoSpin C18 FF offer fast flow of viscosity samples at a low centrifugation speed (1,000 x g).
The following is a comparison of flow of solvents between MonoSpin C18 and MonoSpin C18 FF.

Solvents	Volume	MonoSpin C18	MonoSpin C18 FF
Methanol	500 µL	○	○
Water	500 µL	400 µL	○
Serum*	500 µL	300 µL	○

Testing Conditions

1,000 x *g* 30 sec

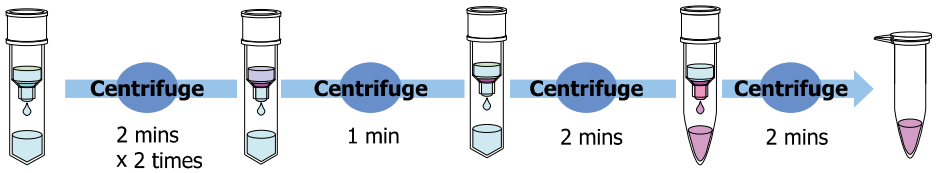
* A supernatant from serum sample was used, and was centrifuged at 10, 000 x g for 1 min.

Purification of Pyridylaminated (PA) Sugar Chain using MonoSpin NH2

Sample Preparation

800 µL sample solution : Add acetonitrile to PA sugar chain sample solution and adjust the acetonitrile final concentration from 90 to 95 %.

Centrifugation
Speed : 2,300 x g



1. Conditioning
Add 500 µL solution mixed with 250 µL 0.1 % formic acid* in water and 250 µL 0.1 % formic acid in acetonitrile
↓ Centrifuge
Add 500 µL solution mixed with 50 µL 0.1 % formic acid* in water and 450 µL 0.1 % formic acid in acetonitrile

2. Adsorption
Add 800 µL sample solution

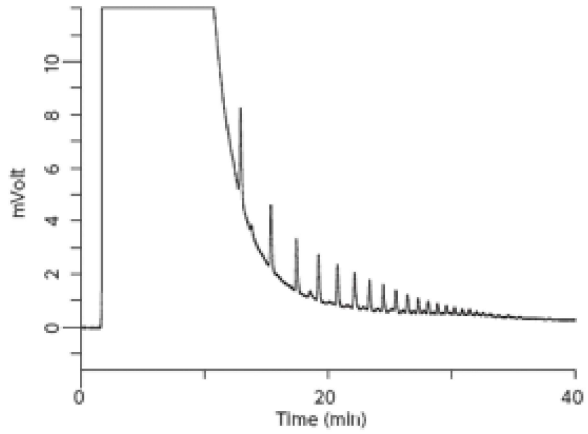
3. Rinsing
Add 500 µL solution mixed with 50 µL 0.1 % formic acid* in water and 450 µL 0.1 % formic acid in acetonitrile

4. Elution
Add 50-800 µL 0.1 % formic acid in 50 % acetonitrile

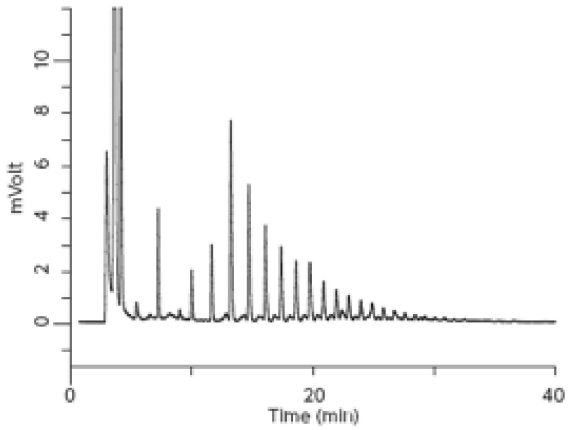
Purified Sample

* Acetic acid or TFA can also be used as an alternative to formic acid.

Without MonoSpin NH2



Purification of PA using MonoSpin NH2



Conditions

Column	: NH_2 Column (5 µm, 250 x 4.6 mm I.D.)
Eluent	: A) H_2O/Acetonitrile = 5/95 0.1 % Formic acid
	B) H_2O/Acetonitrile = 95/5 0.1 % Formic acid
	A/B = 90/10-10 min-90/10-40 min-60/40
Flow Rate	: 1 mL/min
Detection	: FL Em 320 nm, Ex 400 nm
Injection Vol.	: 1.5 µL

Purified PA sugar chain by HILIC mode.
MonoSpin NH2 additionally removes residual fluorescent labeling reagents.

Sample Preparation for LC/MS

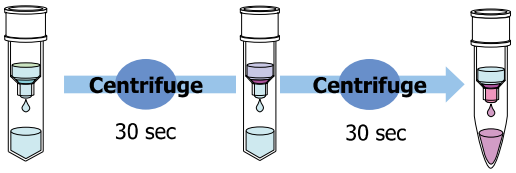
Fractionation of Protein Digests using MonoSpin SCX

MonoSpin SCX provide a rapid and easy fractionation of peptides by stepwise elution using buffers with various salt concentration.

Sample Preparation

500 µL sample solution : First, desalt the peptide solution using MonoSpin C18. Then, dissolve the desalted sample solution with 0.1 % formic acd.

Centrifugation
Speed : 10,000 x g

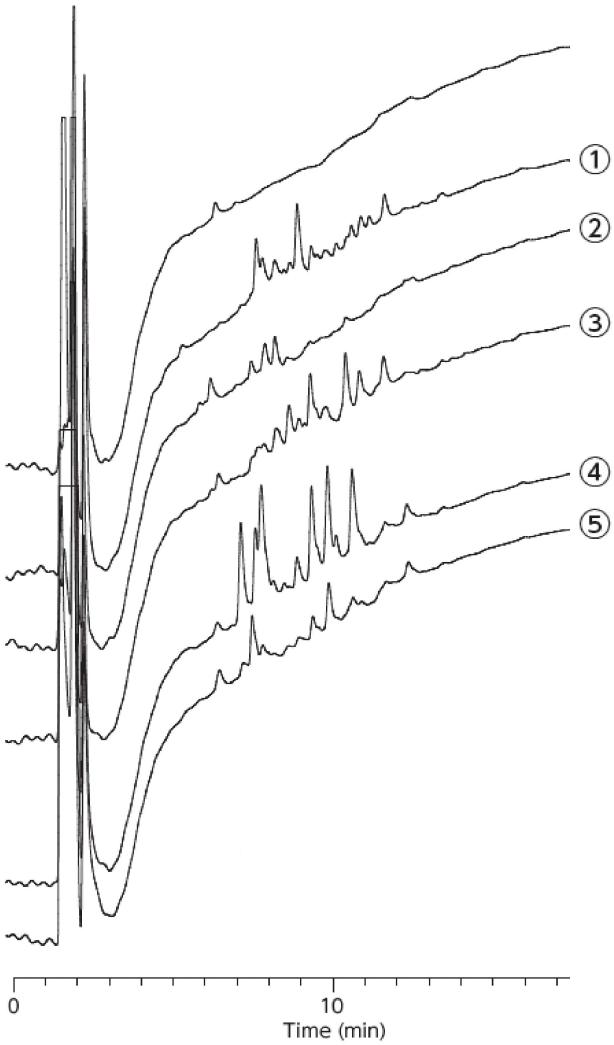


1. Conditioning
Add 300 µL 0.1 % formic acid

2. Adsorption
Add 500 µL peptide sample solution

3. Elution

Sample solution in 0.1 % formic acid



Always replace and attach a new recovery tube whenever adding a new elution buffer

Details of each elution buffer

① 25 mM $HCOONH_4$	200 µL
② 50 mM $HCOONH_4$	200 µL
③ 100 mM $HCOONH_4$	200 µL
④ 500 mM $HCOONH_4$	200 µL
⑤ 1 M $HCOONH_4$	200 µL

* Each elution buffer contains 10 % acetonitrile

Conditions

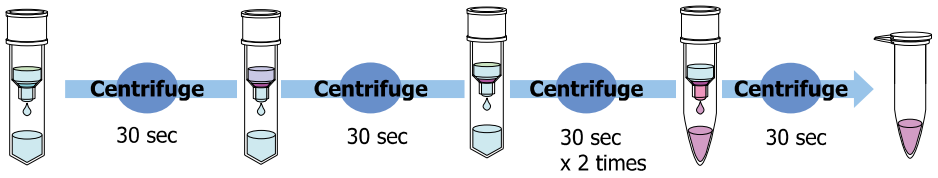
Column : Inertsil ODS-3 (3 µm, 2.1 x 150 mm)
Eluent : A) H_2O (0.1 % HCOOH)
B) Acetonitrile (0.1 % HCOOH)
A/B = 90/10 - 20 min - 50/50
Detection : UV 210 nm
Flow Rate : 0.2 mL/min
Col. Temp. : 40 °C
Injection Vol. : 2 µL

Purification of Paraquat and Diquat using MonoSpin CBA

Sample Preparation

600 µL sample solution :
Add 200 µL urine and and 400 µL 10 mM potassium phosphate buffer (pH 7.0)

Centrifugation
Speed : 10,000 x g



1. Conditioning
Add 200 µL 10 mM potassium phosphate buffer (pH 7.0)

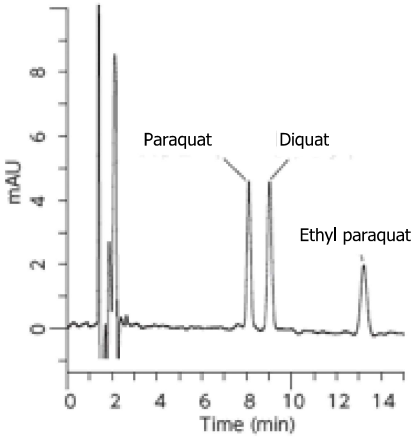
2. Adsorption
Add 600 µL sample solution

3. Rinsing
Add 200 µL 10 mM potassium phosphate buffer (pH 7.0)

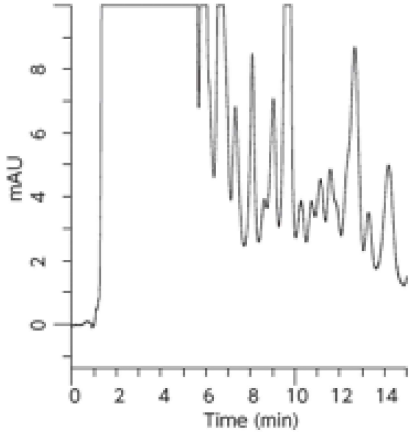
4. Elution
Add 200 µL solution mixed 1 % HCL, 30 % methanol and 69 % water

Purified Sample

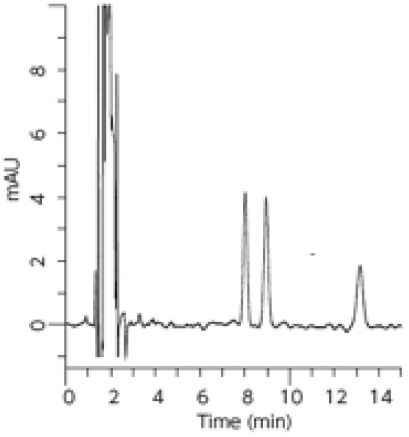
Standard Solution (1 µg/mL)



Urine + Pesticide (1 µg/mL)



Purification using MonoSpin CBA



MonoSpin CBA deliver highly efficient purification of strong basic pesticides such as Paraquat and Diquat.

Conditions

Column	: Inertsil ODS-3 (5 µm, 150 mm x 4.6 mm I.D.)
Eluent	: 0.2 M phosphoric acid, 0.1 M diethyl amine, 7.5 mM IPCC08 (IPCC-0.8, Sodium 1-Octanesulfonate) /Acetonitrile=89/11
Flow Rate	: 1 mL/min
Col.Temp.	: 40 °C
Detection	: PDA 290 nm
Injection Vol.	: 50 µL

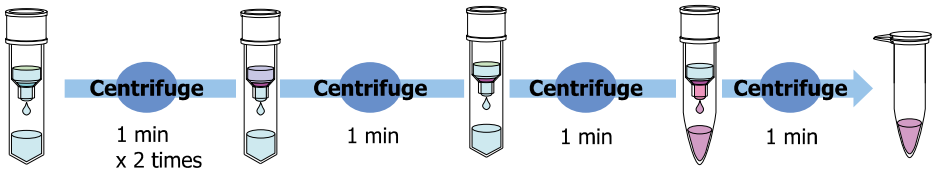
Sample Preparation for LC/MS

Purification of Catecholamines using MonoSpin PBA

Sample Preparation

250 µL sample solution :
Add 200 µL urine or serum and 50 µL 1 M potassium phosphate buffer (pH 8.0 adjust using phosphoric acid)

Centrifugation
Speed : 10,000 x g



1. Conditioning
Add 200 µL 1 % acetic acid
↓ Centrifuge
Add 200 µL 100 mM potassium phosphate buffer (pH 8.0)

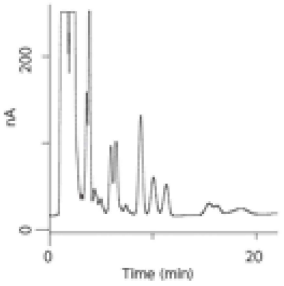
2. Adsorption
Add 250 µL sample solution

3. Rinsing
Add 200 µL 100 mM potassium phosphate buffer (pH 8.0)

4. Elution
Add 200 µL 1 % acetic acid

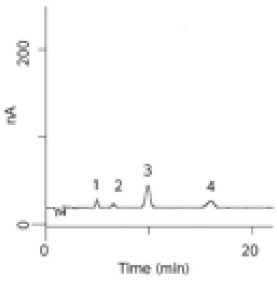
Purified Sample

Without MonoSpin PBA

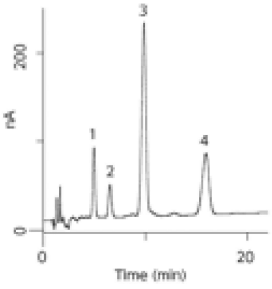


Purification using MonoSpin PBA

500 µL elution



50 µL elution



Conditions

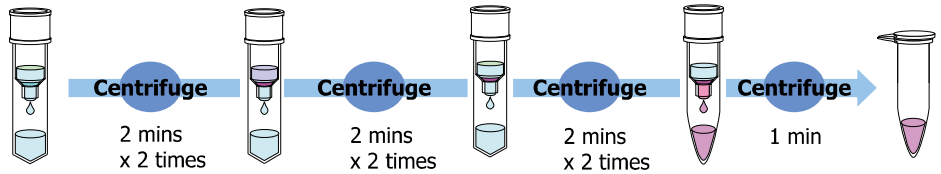
Column : Inertsil ODS-3 (5 µm, 150 mm x 2.1 mm I.D.)
Eluent : 50 mM Phosphate buffer (pH 5.6)
50 mg/L EDTA
600 mg/L IPCC-008
-10 % Methanol
Flow Rate : 0.3 mL/min
Col.Temp. : 35 °C
Injection : 5 µL
Detection : ECD Pulse Mode
Sample : 1. Noradrenaline
2. Adrenaline
3. DHBA
4. Dopamine

Purification of Organic Phosphorous Pesticides in Serum using MonoSpin TiO

Sample Preparation

50 µL sample solution :
Add 10 µL serum sample and 40 µL water

Centrifugation
Speed : 5,200 x g



1. Conditioning
Add 20 µL 0.1 % TFA in 80 % acetonitrile
↓ Centrifuge
Add 20 µL 0.1 % TFA in 50 % acetonitrile

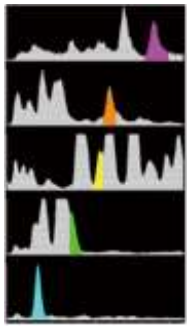
2. Adsorption
Add 50 µL sample solution
↓ Centrifuge
Collect the eluate from the recovery tube & attach a new recovery tube. Then, applythe eluate to MonoSpin TiO again.

3. Rinsing
Add 20 µL 0.1 % TFA in 50 % acetonitrile
↓ Centrifuge
Add 20 µL 0.1 % TFA in 50 % acetonitrile

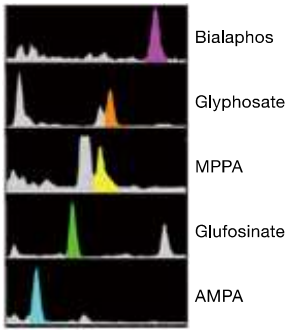
4. Elution
Add 50 µL 2 % ammonia aqueous solution

Purified Sample
Derivatize the purified sample with N-Acetyl-O-methyl and then inject to LC/MS

Without MonoSpin PBA

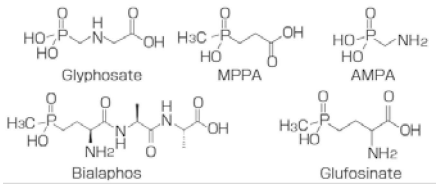


Purification using MonoSpin TiO



Conditions

Column : ODS (150 mm x 2.1 mm I.D.)
Eluent : CH_3OH/20 mM HCO_2NH_4 (pH 3.0) = 15/85
Flow Rate : 200 µL/min
Injection : 5 µL
Detection : SIM
Sample : 1. Bialaphos
2. Glyphosate
3. MPPA
4. Glufosinate
5. AMPA
(1 ppm each)



Rapid Digestion of BSA using MonoSpin Trypsin

Example of Reduction and Alkylation Protocol

1 mg Bovine serum albumin

---- Add 175 µL 500 mM Tris-HCL (pH 8.0) and 8 M urea (Solution 1).

----- Add 25 µL 40 mg/mL dithiothreitol in Solution 1.

----- Incubation at 37 °C for 90 mins

----- Add 50 µL 40 mg/mL iodoacetoamide in Solution 1.

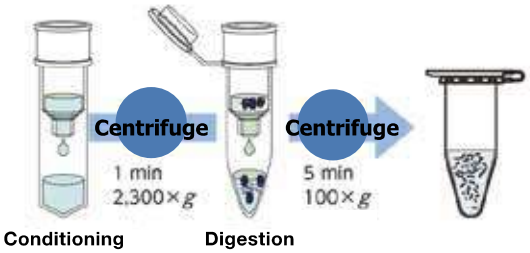
----- Incubation at 37 °C for 30 mins without exposure to light.

250 µL Reduced and alkylated protein

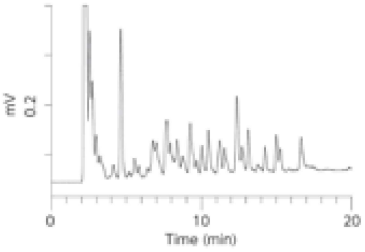
----- Add 50 mM ammonium bicarbonate to make the urea final concentration to 2 M and dilute it to 750 µL

MonoSpin Trypsin

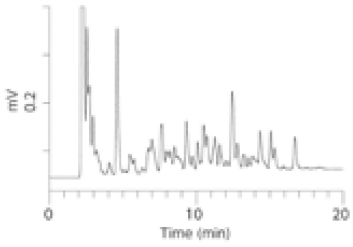
The protocol above is just an example.
Optimize the protocol of preparation of reduced and Alkylated sample depending on the types of proteins.



Incubation at 37 °C
for 10 hours



Protein Digestion at 25 °C for 10 minutes using MonoSpin Trypsin



Conditions

Column	: Inertsil ODS-3 (3 µm, 150 x 2.1 mm I.D.)
Eluent	: A) H_2O (0.1 % HCOOH) B) Acetonitrile (0.1 % HCOOH) A/B = 90/10 - 20 min - 50/50
Flow Rate	: UV 210 nm
Col.Temp.	: 0.2 mL/min
Detection	: 40 °C
Sample	: Digested BSA 2 µL

MonoSpin Trypsin provide rapid and efficient protein digestion at room temperature in 10 mins.

Sample Preparation for LC/MS

List of References

Products	Target Analytes	Sample Matrix	Concentration	Recovery Rate	Detection	Reference No.
MonoSpin C18	amitraz, metabolites	serum	5 ng/mL	95.5, 92.2 %	LC–MS	[1]
	dibudcaine, naphazoline	serum	5 – 10 ng/mL	70.2 – 78.6 %	LC–MS	[2]
	MA, AP, MDA, MDMA	urine	100 ng/mL	96 – 111 %	LC–UV	[3]
	9 cold medicines	serum	5 – 50 ng/mL	2.5 – 73.8 %	GC–MS	[4]
	amphetamines (AP, MA, MDA, MDMA)	urine	5 – 10 ng/mL	84 – 94 %	GC–MS	[5]
	eperison	serum	0.5 ng/mL	92.8 – 96.0 %	GC–MS	[6]
	paraquat, diquat, fenitrothion	serum, urine	25 – 100 ng/mL	51.3 – 106.1 %	GC–MS	[7]
	arsenics	urine	1 ng/mL	91.9 – 106.5 %	GC–MS	[8]
	MAM-2201	blood	1 ng/mL	–	LC–MS/MS	[9]
	a-PVP, a-PBP	urine	1 ng/mL	82 – 100 %	GC–MS	[10]
	a-PVP, a-PBP	hair	0.2 ng/mL	75.5 – 101.5 %	LC–MS	[11]
	Phthalic acid esters	physiological saline	0.2 – 50 µg/L	71.2 – 107.3 %	–	[12]
	<desalting>	digested peptides	–	–	–	[13]
	<desalting>	iTRAQ labeled samples	–	–	–	[14]
	MAM-2201	blood	2.5 – 100 ng/mL	1 ng/mL	–	[15]
	Naringin	grapefruit juice	10 – 500 µM	10 µM	–	[16]
MonoSpin SCX	opiates benzodiazepines, metabolites	urine serum	10 ng/mL 1 – 10 ng/mL	69.2 – 98.9 % 83.3 – 112.3 %	LC–MS	[17]
	<Pre-column fluorescence derivatization>	–	–	–	–	[18]
	<desalting of amino acid>	–	–	–	–	[19]
MonoSpin C18-CX	acidic and basic drugs	urine	1 – 25 ng/mL	65 – 123 %	GC–MS	[20]
	<halogenated compounds>	cells	–	–	–	[21]
MonoSpin C18-AX	amphetamines (AP, MA), opiates, THC	urine	2 – 10 ng/mL	93.1 – 108.1 %	GC–MS	[22]
MonoSpin PBA	Adenosine	urine	6 µM	80 – 113 %	–	[23]

[1] J. Chromatogr., B 867 (2008) 99-104.
[2] J. Chromatogr., B 872 (2008) 186-190.
[3] J. Chromatogr., A 1208 (2008) 71-75.
[4] Chromatographia., 70 (2009) 519-526.
[5] Anal. Chim. Acta., 661 (2010) 42-46.
[6] J. Health Sci., 56 (2010) 598-605.
[7] Anal. Bioanal. Chem., 400 (2011) 25-31.
[8] J. Sep. Sci., 35 (2012) 2506-2513.
[9] Forensic Toxicol., 31 (2013) 333-337.
[10] Forensic Toxicol., 32 (2014) 68-74.
[11] J. Chromatogr., B 942-943 (2013) 15-20.
[12] J Pharm Anal., 1 (2011) 92-99.
[13] Proteomics., 13 (2013) 751-755.
[14] Journal of proteomics., 84 (2013) 40-51.
[15] Forensic Toxicol., 31 (2013) 333-337.
[16] The Journal of Clinical Pharmacology., 54 (2013).
[17] J. AOAC Int., 94 (2011) 765-774.
[18] Biomed. Chromatogr., 26 (2012) 147-151.
[19] Orig Life Evol Bjosph., 43 (2013) 99-108.
[20] J. Sep. Sci., 34 (2011) 2232-2239.
[21] Toxicology., 314 (2013) 22-29.
[22] Forensic Toxicol., 31 (2013) 312-321.
[23] Biosensors and Bioelectronics., 41 (2013) 379-385.

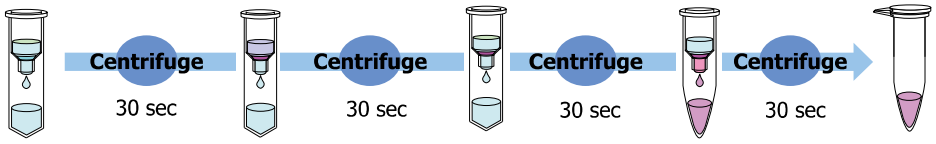
Recovery of Metal Ions using MonoSpin ME

MonoSpin ME is bonded with iminodiacetic acid and optimal for the recovery and purification of metal ions. Specifically, it is excellent for the extraction and purification of trace Pb in blood or urine. Additionally, it is appropriate for removing inorganic divalent cations from sample to prevent ion suppression for LC-MS/MS applications.

Sample Preparation

500 µL 25 µg/mL Cu^{2+}

Centrifugation
Speed : 3,000 x g



1. Conditioning
Add 200 µL water
↓ Centrifuge
Add 200 µL 2N-HNO_3
↓ Centrifuge
Add 400 µL
100 mM CH_3COONH_4
(pH 5.5)

2. Adsorption
Add 500 µL
25 µg/mL Cu^{2+}

3. Rinsing
Add 100 mM
CH_3COONH_4
(pH 5.5)

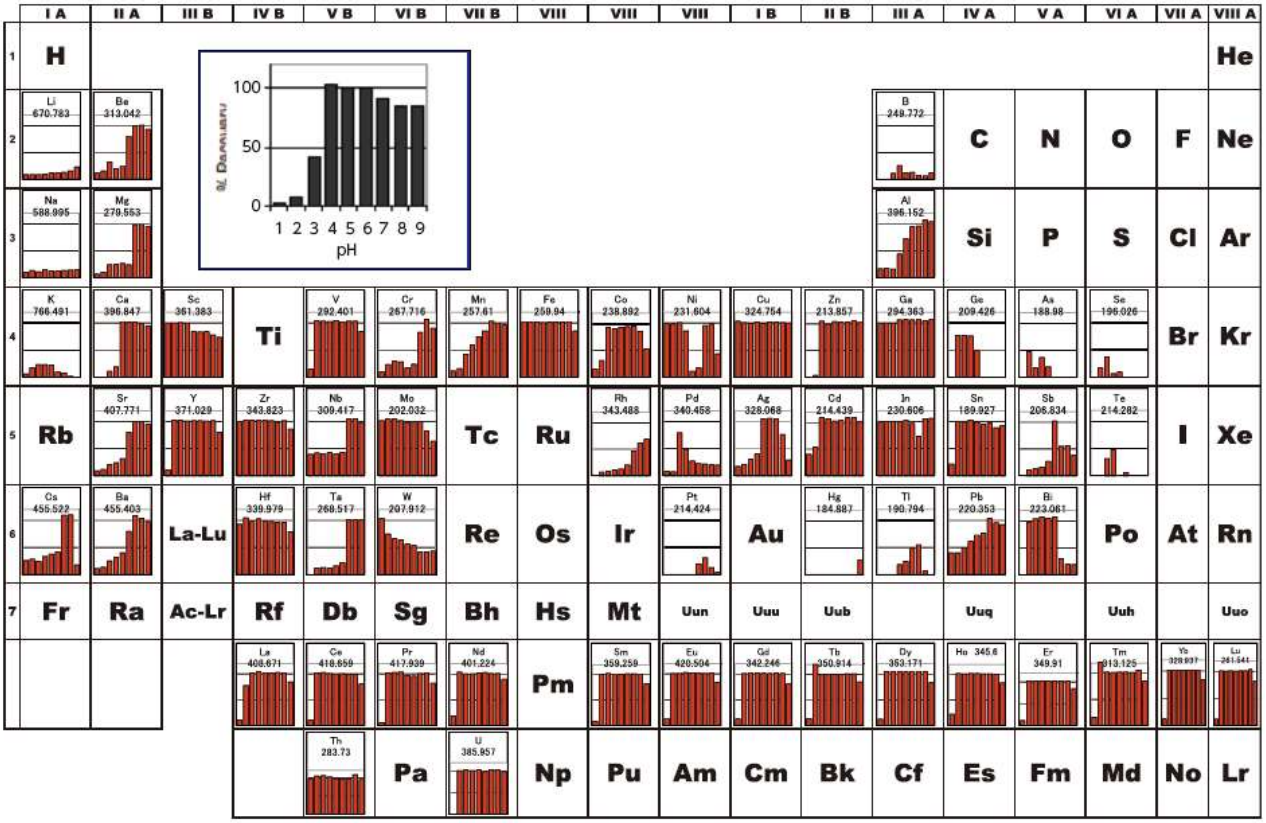
4. Elution
Add 500 µL
2N-HNO_3

Purified Sample

Recovery rate of Cu^{2+} using Zeeman GF-A-AF system

Number of Injections	Volume of solvent introduced	Recovery rate
1	0.8 mL	98 ± 4 %
2	1.6 mL	97 ± 5 %
3	2.4 mL	95 ± 5 %
4	3.2 mL	95 ± 5 %
5	4 mL	94 ± 3 %

Retention Characteristics of Metal Element using Iminodiacetic Acid Functional Groups with Various pH



Sample Preparation for LC/MS

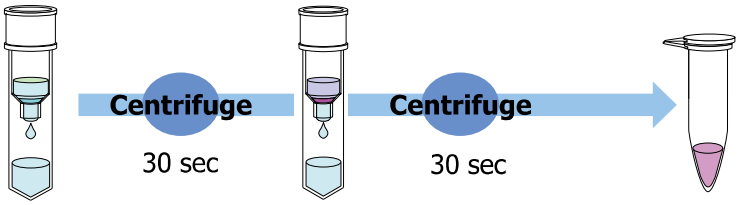
Removal of Phospholipids using MonoSpin Phospholipid

MonoSpin Phospholipid removes more than 90 % of phospholipids from biological samples resulting in eliminating ion suppression in LC-MS/MS analysis. The MonoSpin Phospholipid also removes phospholipids from a serum sample volume of 50 µL.

Sample Preparation

Mix 0.1 % formic acid in acetonitrile with serum (4 : 1) in 2 mL tube. Centrifuge at 10,000 x g for 30 sec. Take the supernatant.

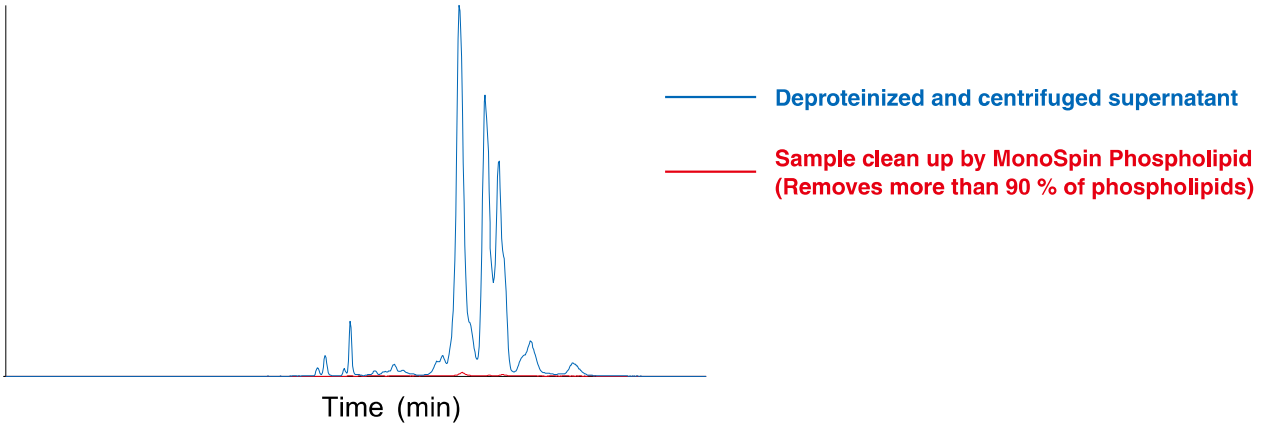
Centrifugation
Speed : 3,000 x g



1. Conditioning
Add 200 µL 0.1 % formic acid in acetonitrile
↓ Centrifuge
Add 200 µL 2N-HNO_3
↓ Centrifuge
Add 400 µL
100 mM CH_3COONH_4
(pH 5.5)

2. Adsorption
Add 500 µL sample solution

Phospholipid-free sample

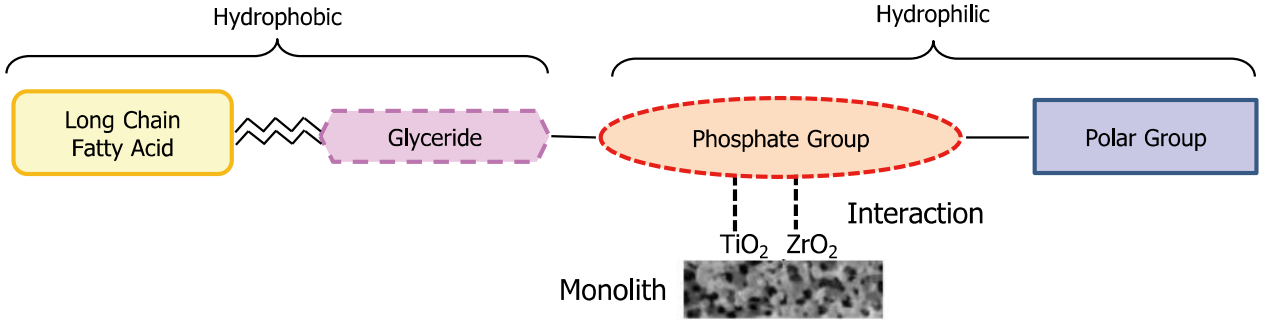


Phospholipid Removal Efficiency of MonoSpin Phospholipid

Retention Mechanism of Phospholipids

Monolith skeletal structure coated with TiO_2 and ZrO_2 selectively interacts with metal oxides and phosphorylated compounds, resulting in removing more than 90 % of phospholipids.

Structure of Phospholipids

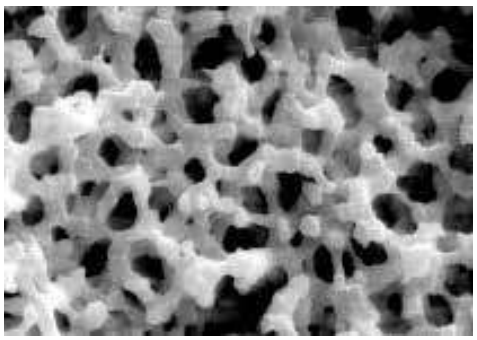


Rapid Purification of Antibodies using MonoSpin ProA and ProG

MonoSpin ProA and MonoSpin ProG are immobilized with protein A or protein G onto a silica monolith offering rapid purification of antibodies. A 96-well plate format is also available for high throughput purification.

Features

The silica is modified with a hydrophilic polymer and then immobilized with either Protein A or Protein G to prevent the adsorption of proteins, resulting in rapid purification and high recovery of antibodies.



Specification

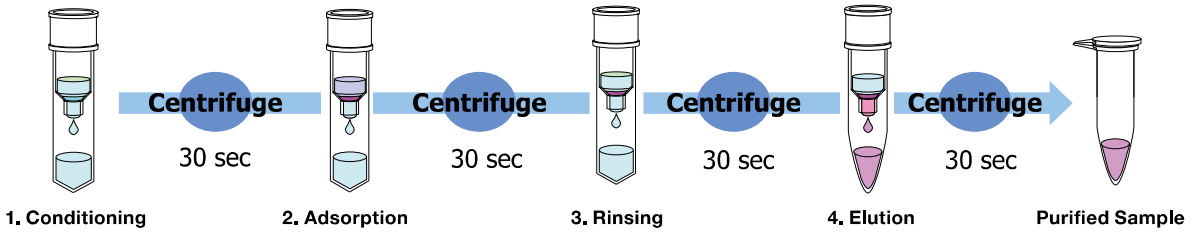
Bonded Phase	Protein A or Protein G
Through-pore	2 µm
Meso-pore Size	60 nm
Disc Size	4.6 O.D. x 1.5 mm
Sample Volume	50 - 500 µL
Recovery Rate	MonoSpin ProA : IgG 90 % (With 400 mg IgG)
	MonoSpin ProG : IgG 90 % (With 300 mg IgG)
Elution Volume	50 µL
Centrifugation speed	2,300 x *g*

Antibody Compatibility Table

Species	Antibody Class	Protein A	Protein G
Human	IgG	Excellent	Excellent
	IgG1	Excellent	Excellent
	IgG2	Excellent	Excellent
	IgG3	–	Excellent
	IgG4	Excellent	Excellent
	IgM	–	–
	IgA	–	–
	IgE	–	–
	IgD	–	–
	Fab	Good	Good
	ScFv	Good	–

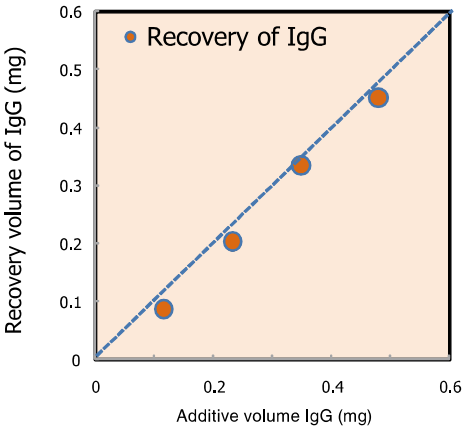
Sample Preparation for LC/MS

Purification of IgG in only Five Minutes using MonoSpin ProA and ProG

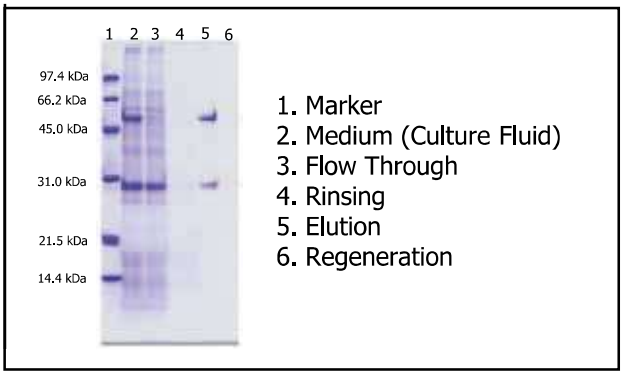


As shown below, the antibody concentrations were determined quantitatively from medium of CHO cells.
The purified antibodies show very less impurities by the results from electrophoresis.

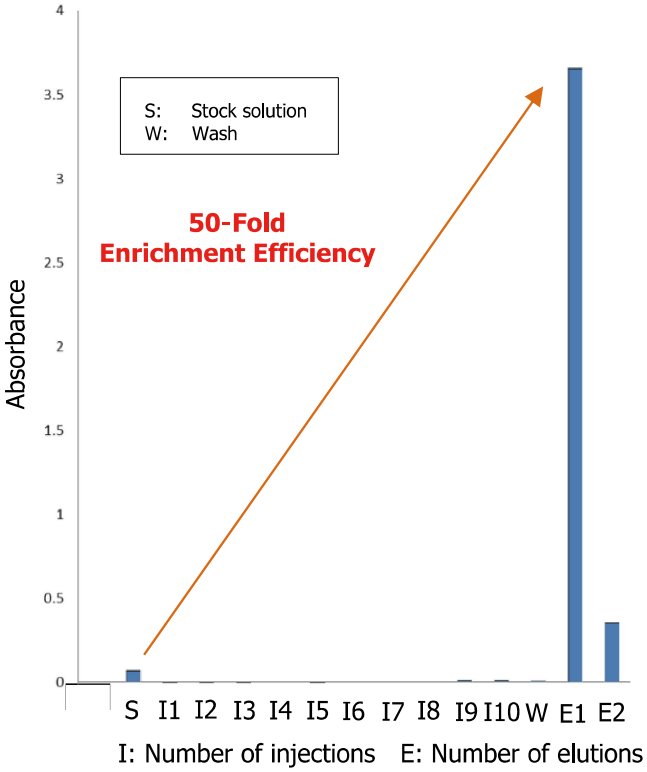
Calibration Curve of IgG Concentration



Results of Recovery by Electrophoresis



Enrichment of Antibody Solution using MonoSpin ProA



500 µL volume of 0.025 mg/mL of human IgG solution was applied to MonoSpin ProA spin column (ten consecutive times).
Then, the elution of IgG concentration was measured with 100 µL elution buffer twice (E1 and E2). The first IgG elution (E1) was 50-fold concentration of the stock solution and showed 90 % recovery of IgG without the loss of IgG.

Comparison of Elution Volume & Recovery Rate with other Brands' Products

MonoSpin ProA only requires 100 µL elution buffer, providing a recovery rate of at least 90 % IgG. On the other hand, other brands' products requires 400 µL of elution buffer with a recovery rate of 70 % IgG.

MonoSpin ProA

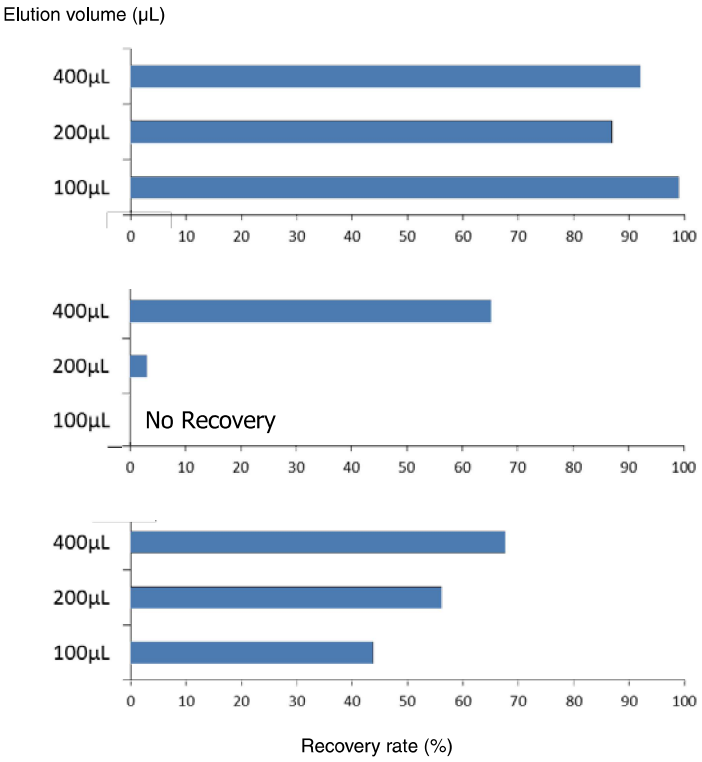
90 % recovery rate of IgG with 100 µL elution

Brand T's Product

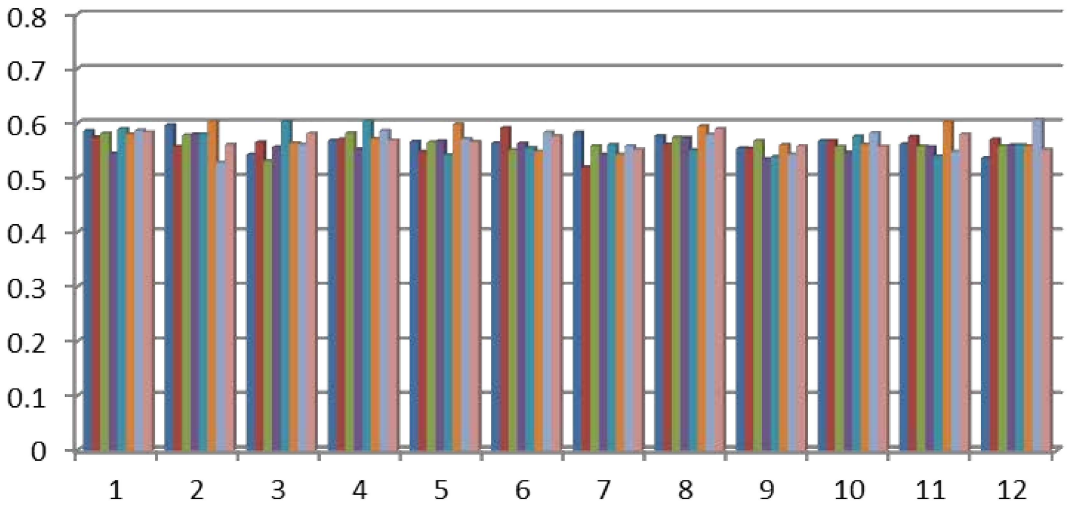
60-65 % recovery rate of IgG with 400 µL elution

Brand G's Product

65-70 % recovery rate of IgG with 400 µL elution



Recovery Rate and Reproducibility of IgG from medium cultured CHO cells with MonoSpin ProA 96-Well Plate



Sample volume : 150 mL
Elution volume : 150 mL
Recovery rate : 90 % (CV 3.1 %)
IgG concentration : 1.3 mg/mL

Sample Preparation for LC/MS

MonoSpin S Type (Small) Columns

・Each MonoSpin S Type (Small) columns are attached with 1.7 mL recovery tubes and 2.0 mL waste tubes.

Description	Qty.	Cat.No.
MonoSpin C18	50 pcs	5010-21700
	100 pcs	5010-21701
MonoSpin C18 FF	50 pcs	5010-21670
	100 pcs	5010-21671
MonoSpin Ph	50 pcs	5010-21733
	100 pcs	5010-21734
MonoSpin C18-AX	50 pcs	5010-21735
	100 pcs	5010-21736
MonoSpin C18-CX	50 pcs	5010-21731
	100 pcs	5010-21732
MonoSpin SAX	50 pcs	5010-21720
	100 pcs	5010-21721
MonoSpin SCX	50 pcs	5010-21725
	100 pcs	5010-21726
MonoSpin NH2	50 pcs	5010-21710
	100 pcs	5010-21711

Description	Qty.	Cat.No.
MonoSpin CBA	50 pcs	5010-21729
	100 pcs	5010-21730
MonoSpin Amide	50 pcs	5010-21727
	100 pcs	5010-21728
MonoSpin PBA	50 pcs	5010-21715
	100 pcs	5010-21716
MonoSpin TiO	50 pcs	5010-21705
	100 pcs	5010-21706
* MonoSpin Trypsin	50 pcs	7820-11300
	100 pcs	7820-11301
MonoSpin ME	50 pcs	5010-21737
	100 pcs	5010-21738
MonoSpin Phospholipid	50 pcs	5010-21698
	100 pcs	5010-21699

* MonoSpin Trypsin must be refrigerated when not in use.

MonoSpin S Type (Small)

Recovery Tube (1.7 mL)

Waste Tube (2 mL)

MonoSpin 96 Well-Plate

Description	Qty.	Cat.No.
MonoSpin 96WP C18	1 pcs	5010-21900
MonoSpin 96WP NH2	1 pcs	5010-21901
MonoSpin 96WP PBA	1 pcs	5010-21902
MonoSpin 96WP SAX	1 pcs	5010-21903
MonoSpin 96WP SCX	1 pcs	5010-21904
MonoSpin 96WP Amide	1 pcs	5010-21905
MonoSpin 96WP CBA	1 pcs	5010-21906
MonoSpin 96WP C18-CX	1 pcs	5010-21907
MonoSpin 96WP C18-AX	1 pcs	5010-21908

MonoSpin L Type (Large)

MonoSpin L Type (Large) Columns

Description	Qty.	Cat.No.
MonoSpin L C18	30 pcs	7510-11320
MonoSpin L SAX	30 pcs	7510-11321
MonoSpin L SCX	30 pcs	7510-11322
MonoSpin L NH2	30 pcs	7510-11323
MonoSpin L CBA	30 pcs	7510-11324
MonoSpin L ME	30 pcs	7510-11325
MonoSpin L Phospholipid	30 pcs	7510-11326

* Each MonoSpin L Type (Large) columns does not include either recovery nor waste tubes.
* Prepare a 50 mL centrifuge tube separately (Ex : Falcon tube).

MonoSpin ProA, MonoSpin ProG

Description	Qty.	Cat.No.
MonoSpin ProA	10 pcs	7510-11310
MonoSpin ProG	10 pcs	7510-11311
MonoSpin ProA 96-Well Plate	1 pc	7510-11312
MonoSpin ProG 96-Well Plate	1 pc	7510-11313

* MonoSpin ProA, ProG must be refrigerated when not in use.

MonoSpin S Type (Small) Trial Kits

・The following trial kits are available for purchase to test the whole range of MonoSpin columns to help the customer to make the best decision on which MonoSpin to use.

Description	Available Phases	Qty.	Cat.No.
MonoSpin Trial Kit 1	C18, TiO, SCX, SAX, 10 pcs each.	10 pcs/4 packs	5010-21740
MonoSpin Trial Kit 2	C18, Amide ,CBA, NH2, 10 pcs each.	10 pcs/4 packs	5010-21741
MonoSpin Trial Kit 3	SCX, SAX, CBA, NH2, 10 pcs each.	10 pcs/4 packs	5010-21742

・MonoSpin Trial Kit 1 : Optimal for drug extraction in biological samples & purification of pesticides.

・MonoSpin Trial Kit 2 : Compatible with both hydrophilic/hydrophobic applications. Optimal for purification of peptide and sugar chains.

・MonoSpin Trial Kit 3 : Optimal for purification of ionic analytes.

Sample Preparation for LC/MS

FastRemover for Protein

Maximizes Sample Yield

FastRemover is a 96-well type filter plate ideal for preparing precipitated protein samples. High-throughput processing of plasma samples is performed simply, accurately, and reproducibly.

Features

- Easy filtration of biological samples.
- Trace analytes can be processed with minimal sample loss due to the low volume design of the elution tip and filter.
- Perfect for processing with automated vacuum instruments.
- High sensitivity analysis is unaffected by contamination from plasticizers or other impurities found in other 96-well plates.
- Removal of microparticle contaminants enables injection to LC/MS/MS directly from the collection plate.

Typical Protocol using FastRemover for Protein

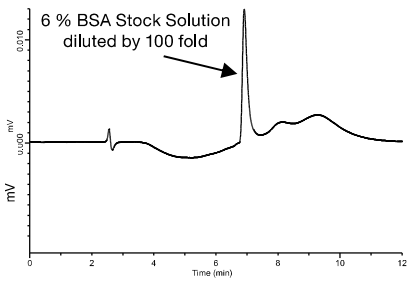
To demonstrate the performance of FastRemover for Protein, a BSA solution was prepared as follows :

Performance of Removal of Proteins

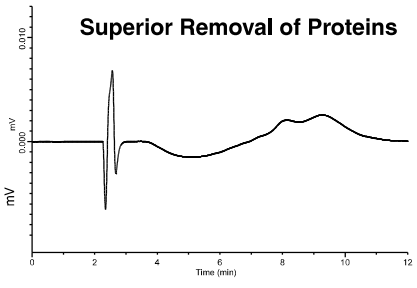
1. 200 µL of plasma is thoroughly mixed in a test tube containing 800 µL of acetonitrile.
2. The FastRemover and collection plate are attached to a vacuum manifold.
3. The BSA sample mixture is loaded into the 96-well plate and vacuum applied above 0.02 Mpa (0.2 Bar) for 2 minutes.

* Methanol can be used as well as a replacement of acetonitrile.

[Standard Sample]



[After filtrated with FastRemover]



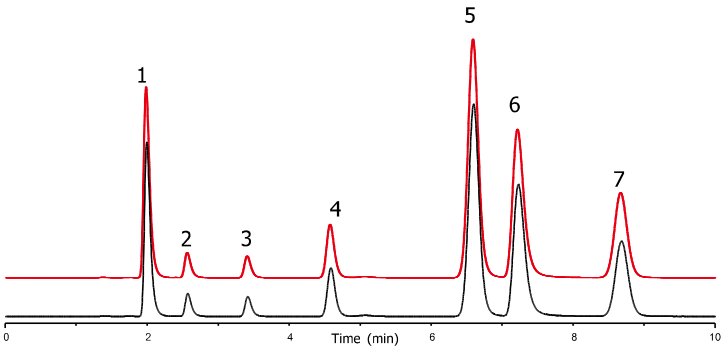
Conditions

Column	: Inertsil WP300 C8 (5 µm, 150 x 2.1 mm I.D.)
Eluent	: A) 0.1 % TFA in CH_3CN B) 0.1 % TFA in H_2O A/B=10/90 - 5 min - 50/50
Flow Rate	: 0.2 mL/min
Col.Temp.	: 40 °C
Detection	: 280 nm
Injection Vol.	: 2 µL

Adsorption Test

A standard mixture containing 7 compounds were analyzed to evaluate potential non-specific adsorption to the plate. As shown in the following chromatograms, FastRemover for Protein provides minimal loss of target samples.

Black : Before treatment with FastRemover
Red : After treatment with FastRemover



Conditions

Column	: Inertsil ODS-3 (3 µm, 150 x 2.1 mm I.D.)
Eluent	: 48 % CH_3CN (0.7 % KH_2PO_4 + 0.17 % SDS, pH 4.5)
Flow Rate	: 0.2 mL/min
Col.Temp.	: 40 °C
Detection	: 230 nm
Injection Vol.	: 1 µL
Samples	1. Acetaminophen 2. Pyridine 3. Phenol 4. Hexobarbital 5. Propranolol 6. Berberine 7. Doxepin

Excellent Non-Specific Adsorption to the Plate

FastRemover for Protein

Description	Qty.	Cat.No.
FastRemover for Protein (0.45 µm) 96-well	1 pc	7820-11001
	5 pcs	7820-11005
FastRemover for Protein (0.20 µm) 96-well	1 pc	7820-11011
	5 pcs	7820-11015

Related Accessories

Description	Qty.	Cat.No.
Vacuum Manifold with shims	1 Set	5010-33101
Sealing Mat for Microplate, WSM-3SX (PTFE/SILCON)	5 pcs	1030-43831
Sealing Tape for Microplate, (Polyolefin)	100 pcs	1065-70002

Sample Preparation for LC/MS

FastRemover for Phospholipid

Rapid and Efficient Removal of Proteins and Phospholipids

The FastRemover for Phospholipid 96-well plate deliver a rapid and effective removal of proteins and phospholipids in plasma and serum samples without sacrificing the recovery of your target analytes.

Features

- Simple & easy protocol to remove proteins and phospholipids.
- High sensitivity analysis is unaffected by contamination from plasticizers or other impurities found in other 96-well plates.
- Removal of microparticle contaminants enables injection to LC/MS/MS directly from the collection plate.
- Removes more than 90 % of phospholipids resulting in eliminating ion-suppression.
- Prolong HPLC/UHPLC column lifetime by removing proteins and phospholipids that can damage your column.

Typical Protocol using FastRemover for Phospholipid

The presence of phospholipids in plasma or serum samples is one of the major problems in LC/MS- (MS) analysis. Phospholipids can build up on your MS system and bleed off the HPLC/UHPLC column, causing ion suppression, shifts in retention time and peak shape and necessitating time consuming column and system maintenance. Use of FastRemover for Phospholipid 96-well plate will eliminate these effects and extend the lifetime of your HPLC/UHPLC column and deliver more predictable/accurate mass spectrometry results.

Easy Protocol by FastRemover for Phospholipid

Step 1 : Solvent Loading

Add 400 µL of 1 % Formic Acid in Acetonitrile into the wells

Step 2 : Sample Loading

Add 100 µL of serum sample into the wells

Step 3 : Mixing

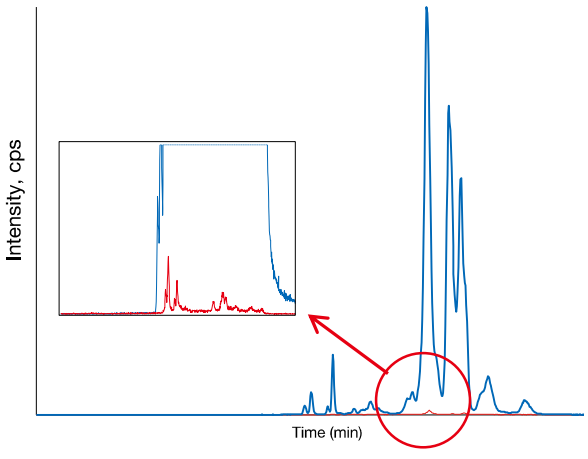
Aspirate the samples up & down couple of times by a manual pipette or an automated pipetting system may be used to ensure complete mixing

Step 4 : Analysis

Collect the extracted sample from the 96-well plate & inject to LC/MS(MS)

*** Traditional Protein Precipitation method :**
Adding acetonitrile to sample and collecting the supernatant layer

RED : FastRemover for Phospholipid
BLUE : * Traditional Protein Precipitation

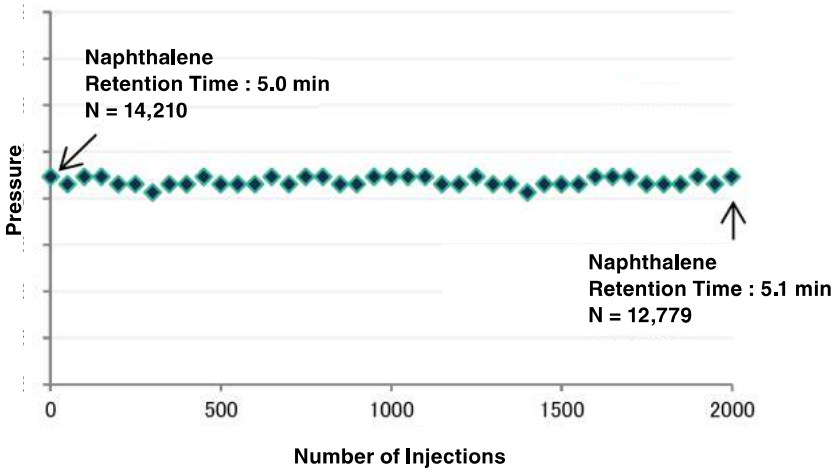


Comparison of Phospholipid Removal between

*** Traditional Protein Precipitation method and FastRemover for Phospholipid**

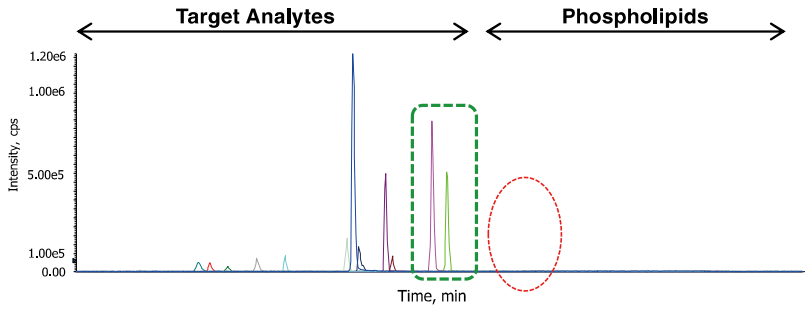
Extend HPLC/UHPLC Column Lifetime

Over the course of multiple injections, phospholipids build up and can lead to reduced column lifetime, showing increase in column back pressure, decrease in column sensitivity and efficiency. The figure below illustrates the removal efficiency of phospholipids, proteins and microparticles by FastRemover for Phospholipid.



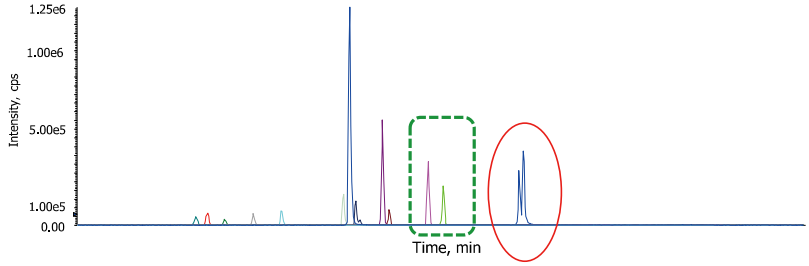
Industry Leading High Recovery for Bioanalysis

As shown below, the FastRemover for Phospholipid 96-well plate delivers a rapid and effective removal of proteins and phospholipids in plasma and serum samples without sacrificing the recovery of your target analytes.



FastRemover for Phospholipid

As shown on the left, not only FastRemover for Phospholipid completely removes phospholipids, but also provide high recovery even for those highly hydrophobic analytes.



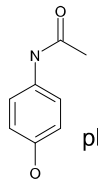
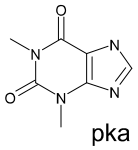
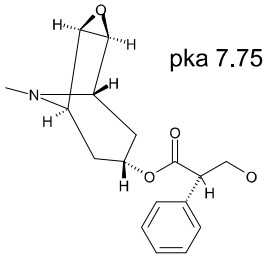
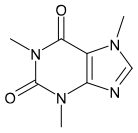
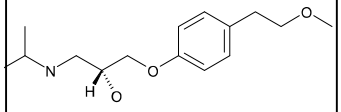
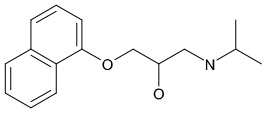
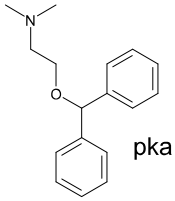
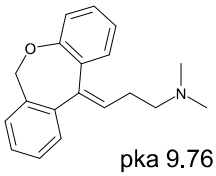
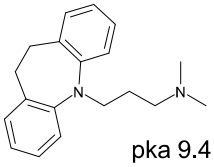
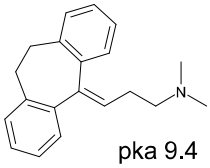
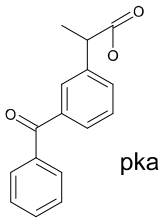
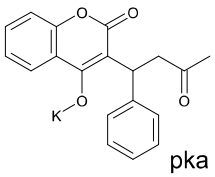
Brand A Phospholipid Removal Plate

Brand A shows adsorption of hydrophobic analytes resulting in poor recovery and elution of phospholipids.

Sample Preparation for LC/MS

Comparison of Recovery Rate using Various Solvents for Deproteinization

The following seven solvents were used to deproteinate a serum sample. As a result, 0.1 % formic acid in 100 % acetonitrile showed the best recovery not only of basic, but also of acidic compounds.

1. Acetaminophen  pka 9.38	2. Theophylline  pka 8.81	3. Scopolamine  pka 7.75	4. Caffeine  pka 10.4
5. Metoprolol  pka 14.09	6. Propranolol(I.S.)  pka 9.42	7. Diphenhydramine  pka 8.98	8. Doxepin  pka 9.76
9. Imipramine  pka 9.4	10. Amitriptyline  pka 9.4	11. Ketoprofen  pka 4.45	12. Warfarin  pka 5.08

Analyte	CH_3OH	CH_3CN	0.1 %HCOOH-CH_3OH	0.1 %HCOOH-CH_3CN	1 %HCOOH-CH_3ON	0.1 %$HCOONH_4$-CH_3OH	1% $HCOONH_4$-CH_3OH
1. Acetaminophen	116.2	92.3	106.9	103.5	102.2	2.7	92.2
2. Scopolamine	75.2	87.4	86.0	87.8	83.6	0.9	68.9
3. Theophylline	104.2	95.7	107.2	96.3	94.1	5.1	94.9
4. Caffeine	97.1	98.2	106.4	97.7	90.6	6.0	98.3
5. Metoprolol	90.7	98.4	89.3	90.8	95.5	1.0	76.8
6. Propranolol	88.2	97.4	92.6	106.4	102.9	1.5	78.3
7. Diphenhydramine	89.1	106.6	92.8	106.3	102.5	2.7	75.7
8. Doxepin	76.9	101.6	86.1	107.2	97.7	1.0	64.7
9. Imipraine	80.9	105.8	82.1	99.9	99.7	1.0	72.8
10. Warfarin	85.4	98.6	84.5	103.1	108.4	1.1	80.6
11. Amitriptyline	60.6	103.9	54.8	86.9	90.9	0.9	73.2
12. Keroprofen	N.D.	39.3	1.4	98.1	108.6	1.9	39.2
13. Diclofenac	8.4	89.2	4.4	102.9	104.9	1.1	67.9

FastRemover for Phospholipid

Description	Qty.	Cat.No.
FastRemover for Phospholipid (0.2 µm)	1 pc	7510-11021

Exosome

EVSecond

Exosome Purification Columns

Recent studies have reported significant roles of extracellular vesicle "Exosome" in development and progression of various diseases including cancer metastasis. Therefore, exosomes are considered as important targets for biomarkers and drugs. However, it remains difficult to isolate high-purity exosomes from biological fluids such as serum. EVSecond is a size exclusion chromatography open column optimized for effective purification of exosomes. Highly-purified exosomes can be easily collected from serum, plasma, or cell culture supernatant.

Features

- Simple gravity-flow handling without ultracentrifugation.
- EVSecond-purified exosomes possess efficient purity for comprehensive miRNA, proteome, and metabolome analysis.
- Exosomes are gently eluted in PBS without structural damage, allowing re-administration experiments of collected exosomes to cells or animals.

Advantages Over Traditional Procedures

- Much higher-purity exosomes can be obtained compared to ultracentrifugation or polymer precipitation methods.
- Unlike immuno-affinity purification using anti-tetraspanin antibodies, whole exosomes can be collected regardless of surface antigen profiles.

Typical Protocol using EVSecond

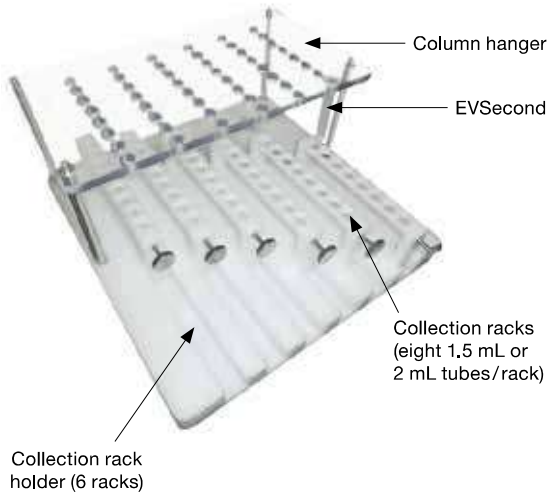
Gravity-flow is applied to each step.

1. Set columns on GL-SPE EXO fraction rack after mixing beads gently and thoroughly.
2. Block beads with 0.22 µm filter-purified FBS.
3. Equilibrate columns with PBS.
4. Load 50-700 µL 0.22 µm filter-purified samples (serum, plasma, or cell culture supernatant).
5. Load PBS and collect appropriate fractions including exosomes.

* Exosome-containing fractions can be identified by western blotting or ELISA experiments detecting tetraspanins (CD9, CD63, CD81, etc.)

GL-SPE EXO Fraction Rack

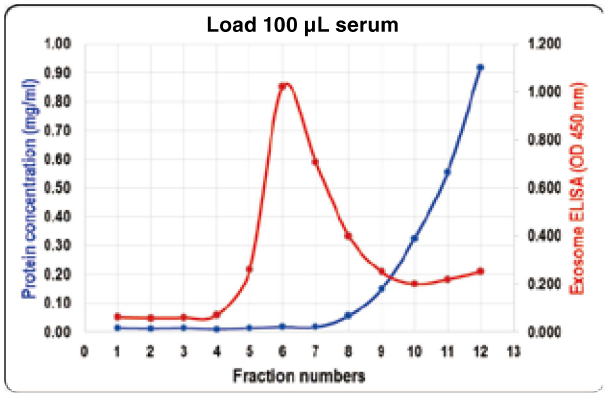
Open column rack optimized for EVSecond.
It helps smooth column handling and fractionation.

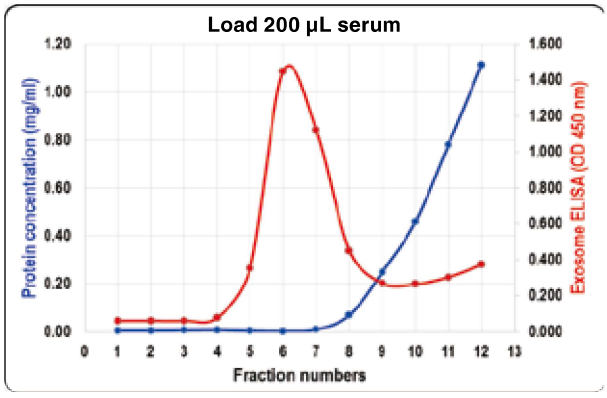


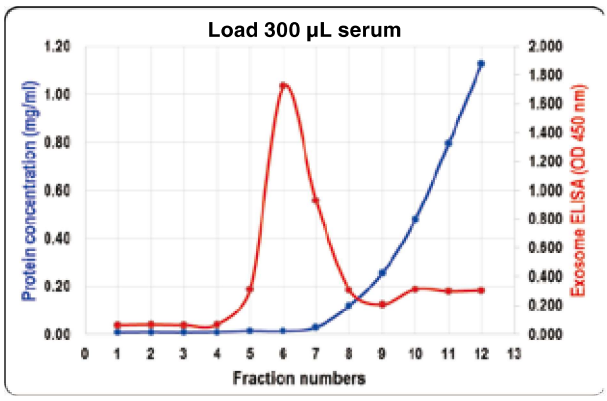
Dimensions : 300 (W) x 300 (D) x 150 (H) mm

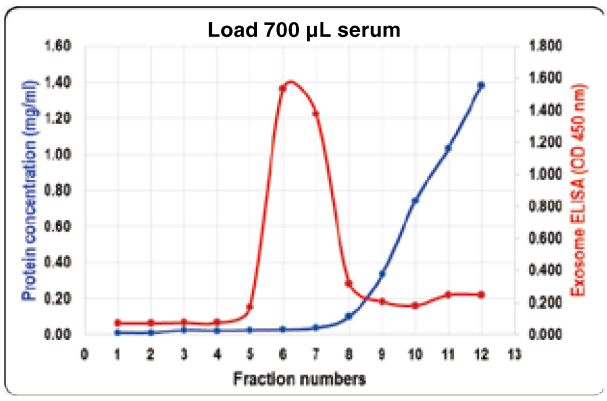
Purification of Exosomes from Human Serum

A large amount of free proteins, metabolites, and nucleotides are involved in serum samples. Insufficient purification of exosomes often causes co-detection of non-exosomal components, leading to incorrect quantification results in omics studies. Exosomes were isolated from 100, 200, 300, or 700 µL of human serum using EVSecond method. Exosomes were clearly separated from serum free proteins such as albumin or immunoglobulins.









(100 µL/fraction)
Red line : CD9-CD9 exosome sandwich ELISA (detecting exosomes)
Blue line : Bradford assay (detecting serum free proteins)
Data provided by Dr. Koji Ueda from Graduate School of Frontier Sciences, The University of Tokyo

EVSecond

Description	Qty.	Cat.No.
EVSecond L70	10 pcs	5010-21395
EVSecond	10 pcs	5010-21390
	25 pcs	5010-21392
GL-SPE EXO Fraction Rack	1 set	5010-50450

EVSecond was developed based on the cooperation from Dr. Koji Ueda from Graduate School of Frontier Sciences, The University of Tokyo.