BIO GENERAL CATALOG

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Proteins & Peptides
Phosphorylation Purification & Enrichment
Low-Molecular Compounds
Extraction & Purification

GL Sciences

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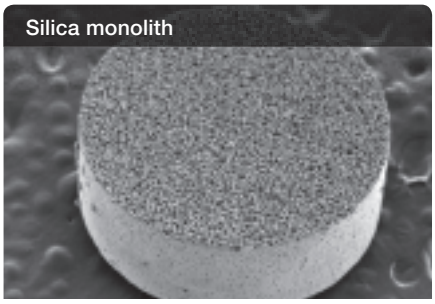
WHAT IS “SILICA MONOLITH” ?

Three-Dimensional Structure ⋯ Large Surface Area

GL Sciences’ silica monolith, created synthetically using ethyl silicate, has a very uniform three dimensional structure that shows excellent reproducibility from batch-to-batch.



Solid Silica Gel Structure ⋯ Effective Enrichment for Small Volume Elution

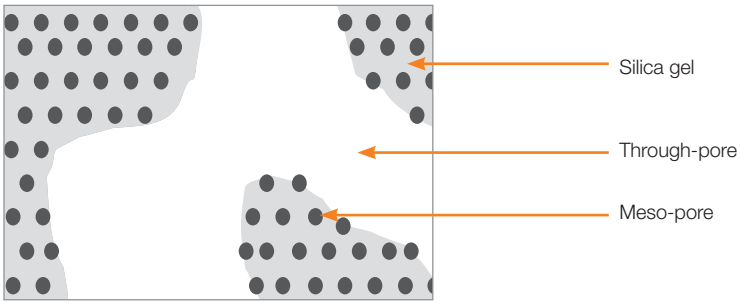
The solid structure of GL Sciences’ silica monolith eliminates the need for frits or filters at the ends of the column, thereby reducing dead volume that might otherwise lead to band broadening or sample recovery. For example, when used in the form of a spin column, samples loaded in 10 µL volume, rinsed, and eluted with 10 µL elution buffer show excellent recoveries.

High Porosity ⋯ Fast Processing

The high porosity of our silica monolith allows high flow rates to be used without loss of resolution or creation of high operating pressure. Even large, delicate analytes, such as long strands of DNA, can be analyzed rapidly without fear of sample degradation.

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.

Silica Monolith Structure

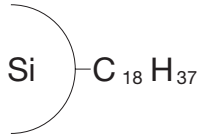


Available Phases of GL Sciences Silica Monolith

Silica gel (Si)

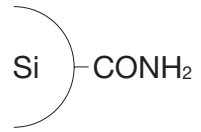
DETAILS	PRODUCTS	APPLICATIONS
Unmodified silica surface provides a polar stationary support used for normal phase chromatography or DNA purification in combination with chaotropic salts	MonoFas I (P.4) MonoFas III (P.6)	Genome DNA purification from legionella bacteria

Octadecyl (C18)



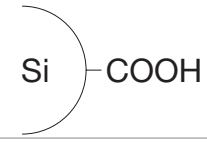
DETAILS	PRODUCTS	APPLICATIONS
Silica monolith bonded with Octadecyl silane groups (ODS), produces a hydrophobic stationary support useful for reversed-phase chromatography	MonoSpin C18 (P.20) MonoTip C18 (P.10)	Drug purification in urine Antihistamine drug extraction from serum

Amide



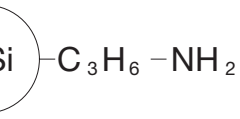
DETAILS	PRODUCTS	APPLICATIONS
Optimal for the extraction of sugar chains and various acidic and basic hydrophilic compounds by HILIC mode	MonoSpin Amide (P.20)	Purification of PA sugar chains

CBA



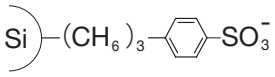
DETAILS	PRODUCTS	APPLICATIONS
Modified with carboxy acid groups combining weak cation exchange. Optimal for the extraction of basic drugs	MonoSpin CBA (P.20)	Basic drug purification in biological samples

Aminopropyl (NH2)



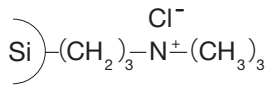
DETAILS	PRODUCTS	APPLICATIONS
Silica monolith bonded with aminopropyl (NH2) groups creates a polar stationary phase useful for sugar analysis or purification. Aminopropyl phase is also good for separating compounds using HILIC mode	MonoSpin NH2 (P.20)	Purification of PA sugar chains

Propyl Benzene Sulfone Acid (SCX)



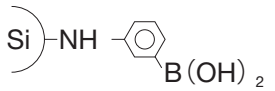
DETAILS	PRODUCTS	APPLICATIONS
Silica monolith bonded with propyl benzene sulfonic acid groups creates a stationary phase that combines strong cation exchange and moderate hydrophobicity. SCX phase is useful particularly well suited for extraction of basic drugs.	MonoSpin SCX (P.20)	Extraction of basic drug

Trimethyl aminopropyl (SAX)



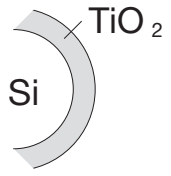
DETAILS	PRODUCTS	APPLICATIONS
Silica monolith bonded with Trimethyl aminopropyl groups creates a stationary phase that combines strong anion exchange and moderate hydrophobicity, particularly well suited for work with acidic drugs	MonoSpin SAX (P.20)	Extraction of acidic drug

Phenylboric acid (PBA)



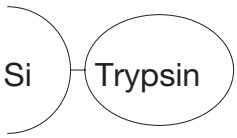
DETAILS	PRODUCTS	APPLICATIONS
Silica monolith bonded with phenylboric acid groups creates a stationary phase particularly well suited for work with compounds containing a catechol structure	MonoSpin PBA (P.20)	Extraction of catecholamines

Titansphere Coating (TiO2)



DETAILS	PRODUCTS	APPLICATIONS
Silica monolith bonded with titanium dioxide creates a stationary phase particularly well suited for work with phosphopeptides and other phospho-group containing compounds	MonoSpin TiO (P.20)	Purification of phosphopeptides Purification of glyphosate (organophosphate pesticides)

Trypsin Fixation (Trypsin)



DETAILS	PRODUCTS	APPLICATIONS
Silica monolith bonded with Trypsin is useful for performing rapid and efficient tryptic digests of protein samples	MonoTip Trypsin (P.8)	Trypsin digestion of proteins

DNA PURIFICATION KITS

DNA Extraction & Purification

MonoFas™ DNA Purification Kit I

MonoFas DNA Purification Kit I purifies DNA from PCR products and agarose gels. Purified DNA can be used for sequencing, cloning/ligation, restriction digests, etc.

Features

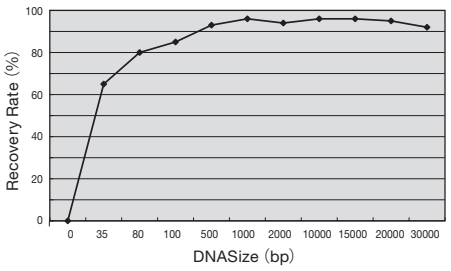
Multiple Roles – Purify DNA from PCR Matrices or Agarose Gels
MonoFas DNA purification Kit I purifies DNA from PCR reaction mixtures and standard or low-melting agarose gels (using TAE or TBE buffers). MonoFas DNA Purification Kit I can be used with centrifugal or vacuum elution methods.

Fast Purification
MonoFAS DNA kits purifies DNA from PCR reaction mixtures in about 4 minutes. DNA can be purified from agarose gels in about 9 minutes. These rapid methods result directly from the high porosity and minimal sample loss of sample volume during elution from the monolith.

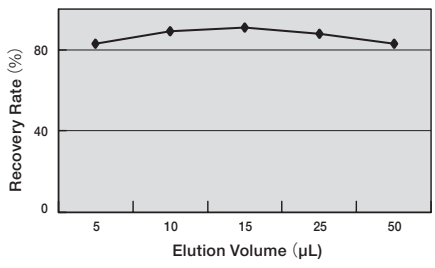
High recovery rates even from sample volumes as low as 10 uL
Unlike other DNA purification products, the monolithic structure of the DNA binding matrix in MonoFas eliminates the need for filters or frits, minimizing elution volume and eliminating recovery losses due to non-specific binding to non-silica frits or filters.

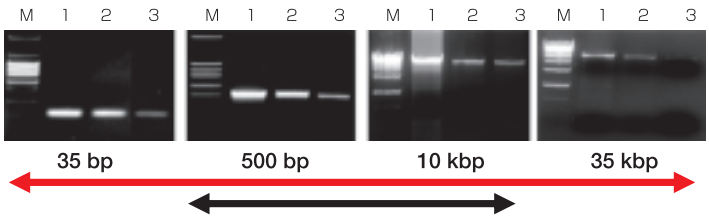
Purify DNA fragmens from 35bp up to 35kbp
MonoFas DNA Purification Kit I purifies double stranded DNA fragments as small as 35 bp and as long as 35 kpb, without damaging or shearing long fragments. Single stranded DNA primers up to 80mer in PCR products are bound by MonoFas.

Recovery Rate by DNA Sizes



Recovery Rate by Elution Volume

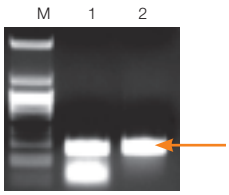




M: pHY Marker
1: PCR Products
2: After purification by MonoFas
3: After purification by competitive products

MonoFas
Competitive Products

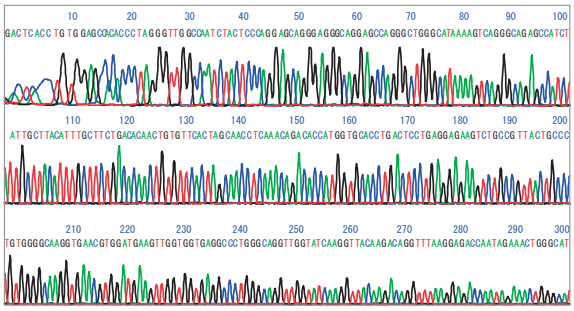
Large quantities of agarose gels can be processed.
MonoFas routinely extracts DNA from up to 1 g of agarose gel at once.



400 bp
M: pHY Marker
1: Unpurified PCR products
2: Purified from agarose gel
(MonoFas DNA Purification Kit I)

Accurate Sequence Analysis

DNA purified with MonoFas products show 98% precision in by flourescence sequencing for fragments over 500bp.



Sample purified by MonoFas DNA Purification Kit I: Cycle sequencing method with Big Dye Terminator v3.1. Manufactured by ABI Model: ABI 3730 Genetic Analyzer

Easy centrifuge on the desk

2 mins: DNA purification from PCR products

7 mins: DNA purification from agarose gel

Steady rotation
> 6,200 rpm (+/- 20%)

Constant centrifuge acceleration
> 2,000 × g (19,600 m/s^2)

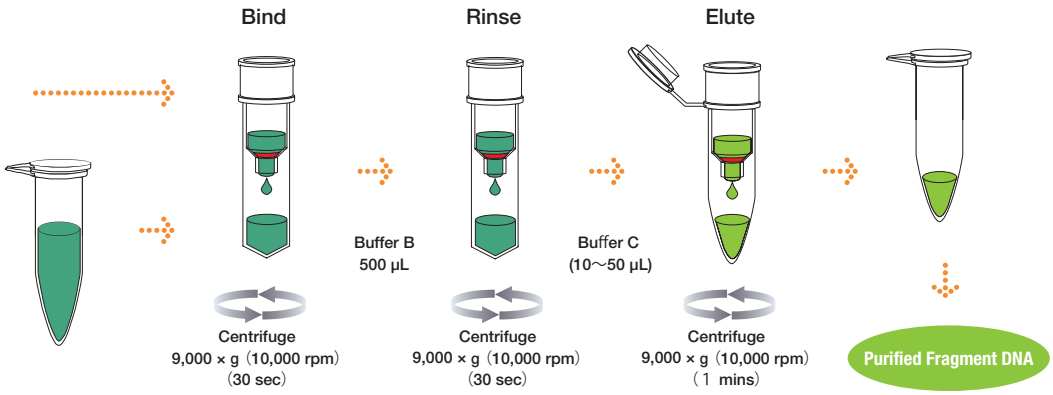
How to Operate

1.Purification of PCR products

Load the PCR products and Buffer A (10 times the volume of PCR products) into the MonoFas spin column

2.Extraction from agarose gel

Add the Buffer A (equal volume as the agarose gel), dissolve for 5 mins at 60 ºC then load it into the MonoFas spin column



Specifications

Description	Purification from PCR products	Extraction from agarose gel
Time	4 mins	9 mins
Maximum DNA Binding Amount	<10 µg	<10 µg
Maximum Agarose Gel Throughput	—	< 1 g
Minimum Elution Amount	10 µL	10 µL
Column Volume	1 mL	1 mL
Processable DNA Range	35 bp - 35 kbp	35 bp - 35 kbp
Recovery Rate	>85 % (100 bp - 5 kbp)	>80 % (100 bp - 5 kbp)
	>60 % (5 kbp - 35 kbp)	>50 % (5 kbp - 35 kbp)
Primer Removal Percentage	95 %	—

MonoFas™ DNA Purification Kit I Part Number

Description	Quantity	Cat.No.
MonoFas DNA Purification kit I	50 times	5010-21530
	100 times	5010-21531
	250 times	5010-21532
Buffer A	50 mL	5010-21506
Buffer B	21 mL	5010-21509
Buffer C	10 mL	5010-21508
Spin Column	100 pcs	5010-21541

Due to the exporting regulations (IATA), ethanol cannot be shipped. Please prepare ethanol before use to complete Buffer D-3.

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DNA PURIFICATION KITS

Plasmid DNA Extraction from E.coli

MonoFas™ Plasmid Extraction Kit III

Rapid Purification

MonoFas Plasmid Extraction Kit III is designed to purify plasmid DNA from E. Col cultures. The extracted plasmid DNA can be used without further purification for sequence analysis, restriction digestion, cloning/ligation, etc.

Features

Rapid Plasmid Purification

Only 8 minutes are required to purify plasmid DNA using MonoFas Plasmid Extraction Kit III.

Highly Purified Plasmid DNA

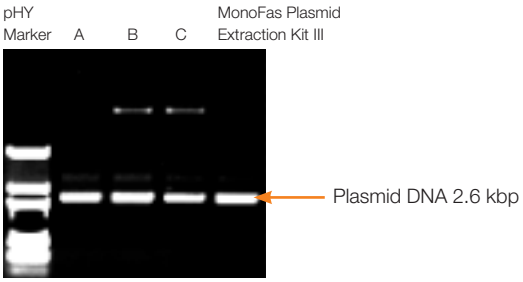
By exploiting the unique features of GL Sciences' monolithic silica, MonoFas Plasmid Extraction Kit III produces highly purified DNA, rapidly. No ethanol precipation or chloroform extraction is needed following extraction..

BAC Clone purification

With the large surface of the Silica Monolith, BAC clone can be also purified.

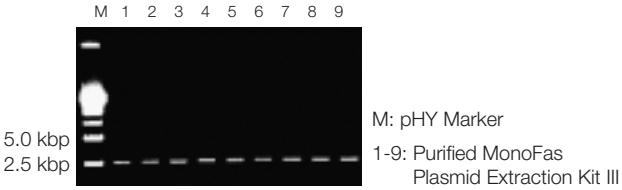
Comparison against market available plasmid kit

MonoFas Plasmid Extraction Kit III extracts purer plasmid DNA compared to other market available plasmid kits. This is due to the monolith silica structure that does not need extra pressure and the large surface areas for better nucleic acid adsorption.

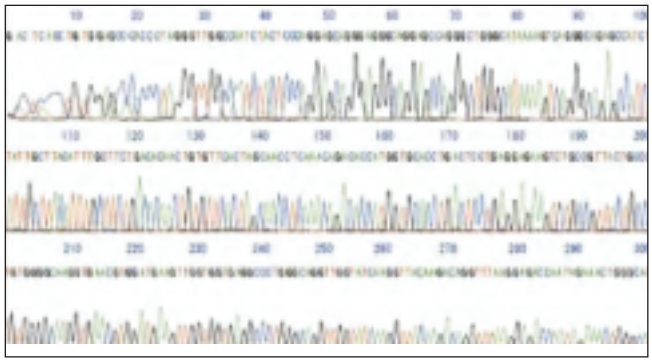


Stable Recovery Rate

Due to the strict lot control of silica monolith, the same amount of Plasmid can be extracted from the same E. Coli culture solution. The picture below shows the extraction of 2.6 kb low copy Plasmid in JM 109 bacteria coli and the recovery rate.



Accurate Sequence Analysis

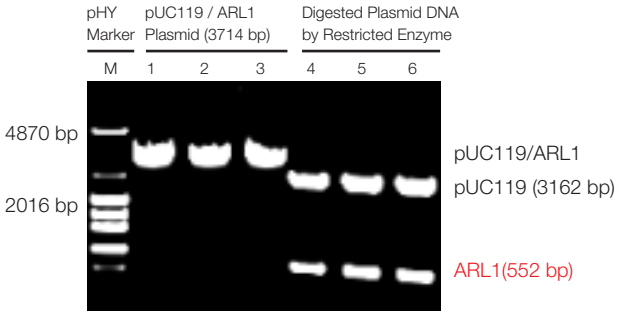


Sample: MonoFas Plasmid Extraction Kit III was used to purify human genome DNA from a Takara PCR Kit reacion mixture. Sequence data analyzed with ABI Prism 3730xl Genetic Analyzer.

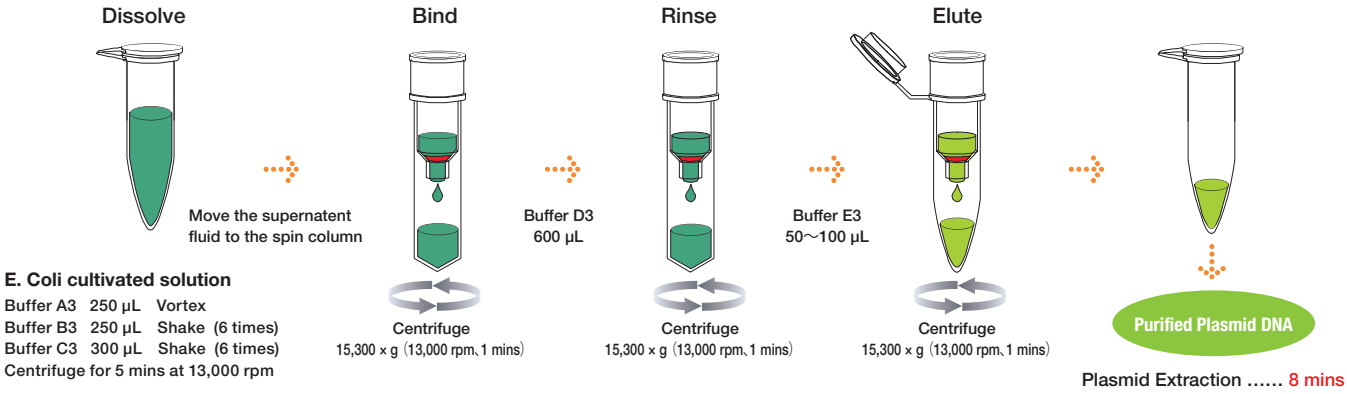
Application

Extract plasmid DNA containing the ARL1 gene from E. coli (DH 5 α) using MonoFas Plasmid Extraction Kit III (Lane 1-3)

Extracted plasmid digested with EcoR I - Hind III, confirming target gene (ARL1: 581bp) (Lane 4-6)



How to Operate



Plasmid Extraction ..... 8 mins

Specifications

Description	Volume
Cultivated Solution Throughput	1 - 3 mL
High - Copy-Plasmid	15 µg/mL culture
Low - Copy-Plasmid	5 µg/mL culture
Recommended Elution Volume	50 - 100 µL
DNA Purity (O. D. 260 / 280 mm)	1.7 - 1.9
Column Volume	1 mL

MonoFas™ Plasmid Extraction Kit III Part Number

Description	Quantity	Cat.No.
MonoFas Plasmid Extraction Kit III	50 times	5010-21533
	100 times	5010-21534
	250 times	5010-21535
Buffer A3	50 mL	5010-21515
Buffer B3	50 mL	5010-21516
Buffer C3	50 mL	5010-21517
Buffer D3	21 mL	5010-21521
Buffer E3	10 mL	5010-21519
Rnase A Buffer	500 µL	5010-21520

Due to the exporting regulations (IATA), ethanol cannot be shipped.
Please prepare ethanol before use to complete Buffer D-3.

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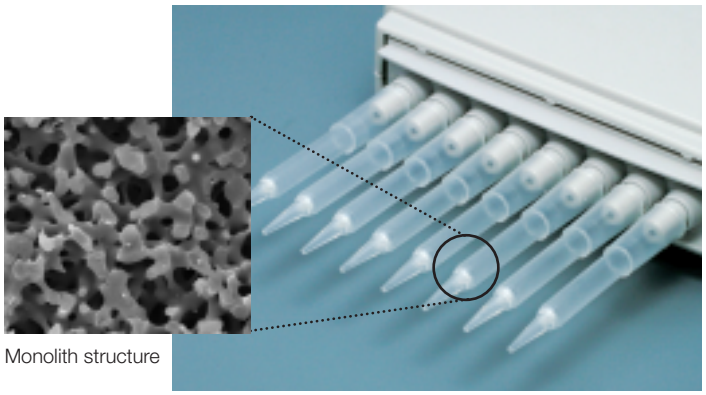
PROTEINS & PEPTIDES

On-Column Trypsin Digestion Simple and Fast

MonoTip™ Trypsin

Only 20 minutes to digest trypsin MonoTip Trypsin is a sample preparation tip with Silica Monolith consisting continuous through-pores with protein digestive enzyme, Trypsin, fixed.



Monolith structure

Features

More efficient than solution digests

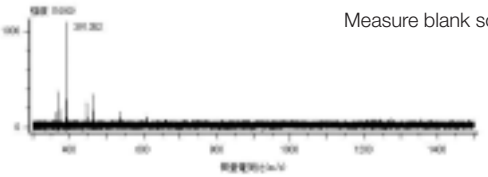
High density bonding of trypsin on GL Sciences' silica monolith results in faster protein digestion than occurs in traditional solution digests. MonoTip Trypsin tips contain ~ 100u of trypsin per tip, allowing digestion of highly concentrated protein samples. Solution protein digests with trypsin typically requires up to 10 hours at 37ºC; however, this new technology yields equivalent digestion efficiency in only 20 minutes at room temperature.

MonoTip Trypsin Tips have a long shelf life.

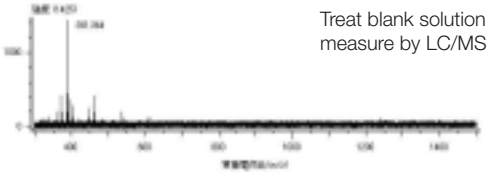
MonoTip Trypsin can be stored without detriment for two weeks at room termperature and up to 1 year if refrigerated.

No Trypsin Self-Digestion

By bonding trypsin to the monolilth silica surface, each attached enzyme is incapable of digesting neighboring enzymes, so digestion efficiency is dramatically improved. MonoTip Trypsin tips are TPCK treated to eliminate chymotrypsin interference.



Measure blank solution by LC/MS



Treat blank solution by MonoTip Trypsin and measure by LC/MS

How to Operate

Conditioning

Aspirate and discharge twice the 100 µL of 50 mM Ammonium Bicarbonate Water Solution

Protein

Reduction/Alkylation

MonoTip Trypsin

Pipetting — 20 times at room temperature

Rest — for 15 mins at room temperature

Trypsin digested protein

Existing bath method: 10 hours at 37 °C

MonoTip Trypsin: 20 minutes at room temperature (20 °C)

*It might be difficult to digest samples such as histogenous cells in a short time.

Reference

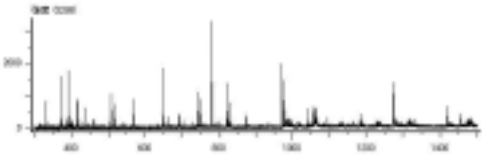
High-throughput protein digestion by trypsin-immobilized monolithic silica with pipette-tip formula.

Ota S et.al J Biochem Biophys Methods. 2007 Feb 23;70(1):57-6

Application

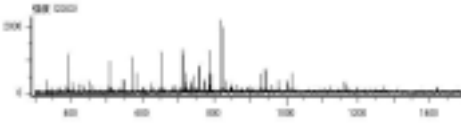
β-Casein, cow whole blood albumin and transferrin were digested by MonoTip Trypsin and analyzed by LC/MS(/MS).

Cow whole Blood Albumin



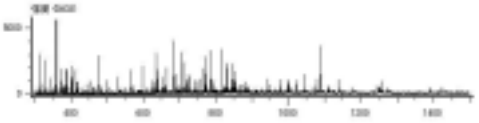
Mass charge ratio

Casein(phosphopeptid protein)



Mass charge ratio

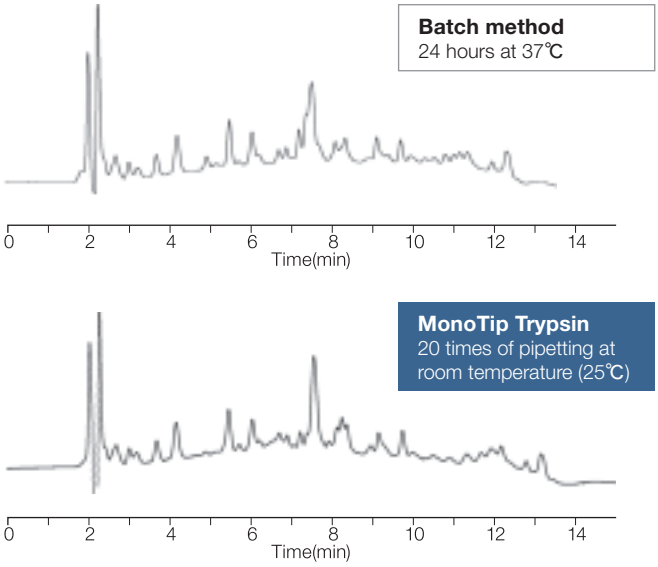
Transferrin(glycoprotein)



Mass charge ratio

Comparison between batch method (existing method) and MonoTip Trypsin

Sample: Ovalbumin (1 mg/mL)

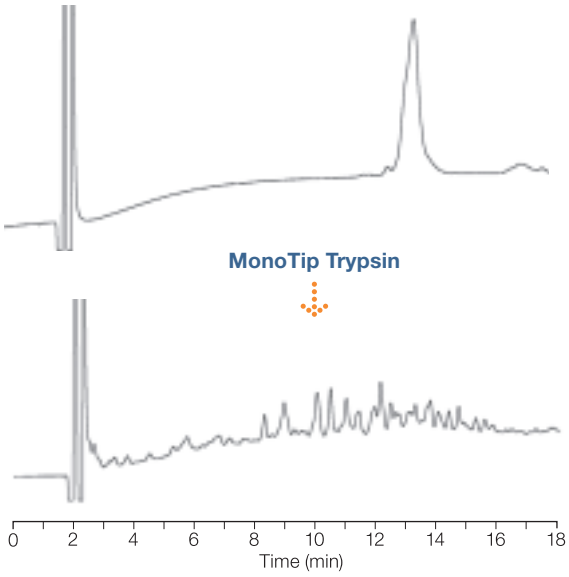


Conditions

Column: Inertsil WP300 C8 (150 × 4.6 mm I.D.)
Eluent: A: H_2O (0.1% TFA) B: CH_3CN (0.1% TFA)
A/B = 90/10-(10 min)-40/60
Flow Rate: 1 mL/min
Detection: UV 210 nm

Highly concentrated protein digestion

Deducted/Alkylized transferrin(100 µg)



Conditions

Column: Inertsil WP300 C8 (150 × 4.6 mm I.D.)
Eluent: A: H_2O (0.1 %TFA) B: CH_3CN (0.1% TFA)
A/B = 90/10-(20 min)-40/60
Flow Rate: 1 mL/min
Detection: UV 210 nm

Specifications

Description	Specification
Time	20 minutes
Sample Volume	20 ～ 200 µL
Tip Volume	200 µL
Derivation	Cow Pancreas
Organic Solvent Resistence	Acetonitrile less than 20 %
Packing Material	Silica Monolith
Through-Pore Diameter	10 ～ 20 µm
Meso-Pore Diameter	300 Å (30 nm)
Surface Area	100 m^2/g
Chemical Bonding	TPCK Treated Trypsin

MonoTip™ Trypsin Part Number

Description	Quantity	Cat.No.
MonoTip Trypsin	24 pcs	5010-21012
	96 pcs	5010-21010

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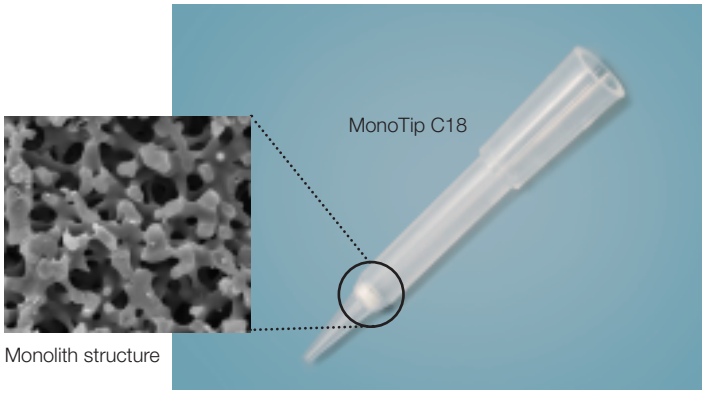
PROTEINS & PEPTIDES

Desalting & Enrichment of Proteins and Peptides

MonoTip™ C18

Designed for Desalting & Enrichment of Proteins and Peptides

MonoTip C18 pipette tips contain silica monolith bonded with C18 (ODS) groups. This formulation enrichment and demineraliztion of proteins and peptides by simple reverse phase binding mechanisms.



Features

Easy to operate

Pipetting protein / peptide samples is typically easier and less time consuming than using tradition SPE methods.

Efficient even with dilute samples

MonoTip C18 is unusually efficient for peptide and protein samples in the pmol to nmol range with molecular weight up to 40 kDa, in loading volumes of up to 200uL.

How to Operate

MonoTip C18

Conditioning

1. 100% Acetonitrile Aspirate/Discharge Twice
2. 20% Acetonitrile (0.1% TFA)

Bind — Aspirate and Discharge sample 6 times
Add 0.1% TFA to the sample

Rinse — 0 - 20% Acetonitrile (0.1% TFA)
Aspirate – Discharge 3 times

Elute — 60% Acetonitrile (0.1% TFA)
Aspirate - Discharge 5 times

Reference

"Simultaneous determination of ten antihistamine drugs in human plasma using pipette tip solid-phase extraction and gas chromatography/mass spectrometry"

Hasegawa C et al., Rapid Commun. Mass Spectrom (2006);20: 537-543

"Rapid demonstration of diversity of sulfatide molecular species from biological materials by MALDI-TOF/MS

Kyogashima M et al., Glycobiology (2006); 16(8),719-728

Simultaneous determination of methamphetamine and amphetamine in human urine using pipette tip solid-phase extraction and gas chromatography-mass spectrometry.

Kumazawa T. et al. J Pharm Biomed Anal. 2007 Jan 8;

Establishment of a quantitative, qualitative, and high-throughput analysis of sulfatides from small amounts of sera by matrix-assisted laser desorption ionization-time of flight mass spectrometry.

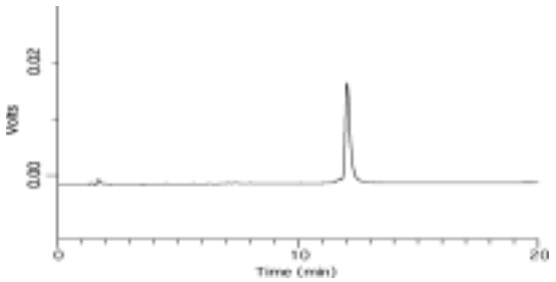
Li G et.al Anal Biochem. 2007 Mar 1;362(1):1-7

Application

Minimal loss of proteins during desalting

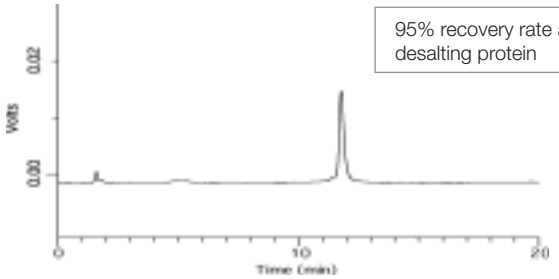
Sample: Cytochrome C in Tris-HCl (pH 7.4) 100 nM NaCl (0.6 mg/mL)

Before desalting



MonoTip C18

After desalting



95% recovery rate after desalting protein

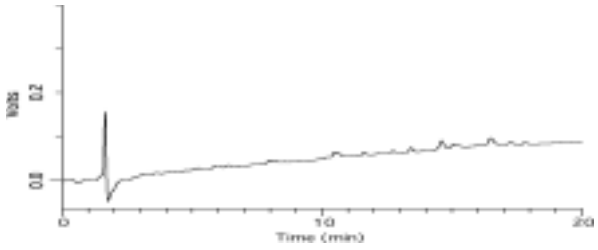
Conditions

Column: Inertsil WP300 C18 (150 × 2.1 mm I.D.)
Eluent: A: H_2O (0.1 %TFA)
B: CH_3CN/H_2O=90/10 (0.1%TFA)
A/B = 80/20-(20 min)-40/60
Flow Rate: 0.3 mL/min
Injection: 5 µL
Detection: UV 280 nm

Effective sample enrichment

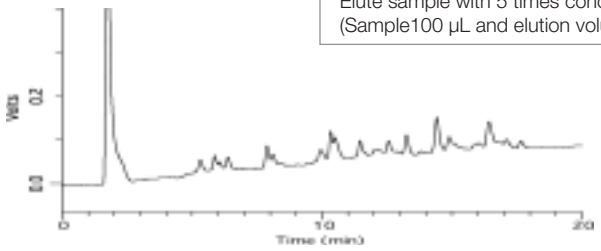
Sample: B-Casein Tryptic Digest (0.1 mg/L)

Direct sample injection



MonoTip C18

Direct sample injection



Elute sample with 5 times concentration (Sample100 µL and elution volume 20 µL)

Conditions

Column: Inertsil WP300 C18 (150 × 2.1 mm I.D.)
Eluent: A: H_2O (0.1 %TFA)
B: CH_3CN/H_2O=90/10 (0.1 %TFA)
A/B = 90/10-(20 min)-40/60
Flow Rate: 0.3 mL/min
Injection: 5 µL
Detection: UV 210 nm

Specifications

Description	MonoTip C18
Sample volume	20 ~ 200 µL
Sample treatment concentration	pmol ~ nmol order
Sample loading concentration	100 µg (Angiotensin II)
Tip volume	200 µL
Functional group	Octadecyl
Organic solvent resistance	Acetonitrile 100%
Packing material	Highly pure silica monolith
Through-pore diameter	10 ~ 20 µm
Meso-pore	200 Å (20 nm)
Surface area	200 m^2/g

MonoTip™ C18 Part Number

Description	Volume	Quantity	Cat.No.
MonoTip C18	200 µL	24 pcs	5010-21002
		96 pcs	5010-21000

"Based on monolithic technology, Merck KGaA, Darmstadt, Germany"

PURIFICATION & ENRICHMENT OF PHOSPHOPEPTIDES

Phosphorylated Protein Research

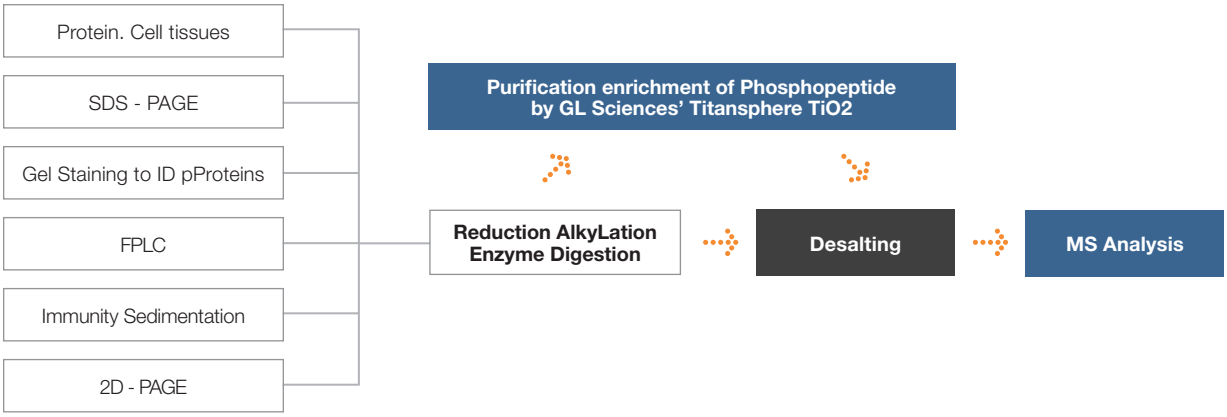
Protein phosphorylation is recognized as a fundemental process which regulates cell differentiation, growth, and migration. Analyzing protein phosphorylation is complicated by the low concentration of any given phosphoprotein and any one time, and the relatively low ionization efficiency of phosphopeptides in MS analysis. 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 (TiO2 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?

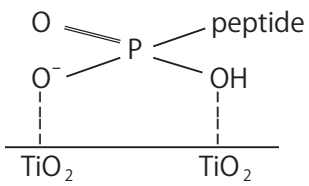
Titanium Dioxide exists in three crystaline forms, known as rutile, anatase, and brookite. Rutile and Anatase forms are the most common and most useful for phosphopeptide enrichment, and the ratio of rutile form to anatase form has significant implications for applicabililty to enrichment of phosphopeptides. GL Sciences' manufacturing technique for it's phosphopeptide enrichment products produces a highly spherical bead with the optimum ratio of crystal forms of TiO2. The primary reasons the GL Sciences' Pho-TiO and MonoTip producs show superior performance is a direct result of the unique formulation of our titanium dioxide beads.

Basics of Phophopeptide Analyses by MS



Principal of Phosphopeptide Enrichment using GL Sciences' Phos-TiO Sample Enrichment Products

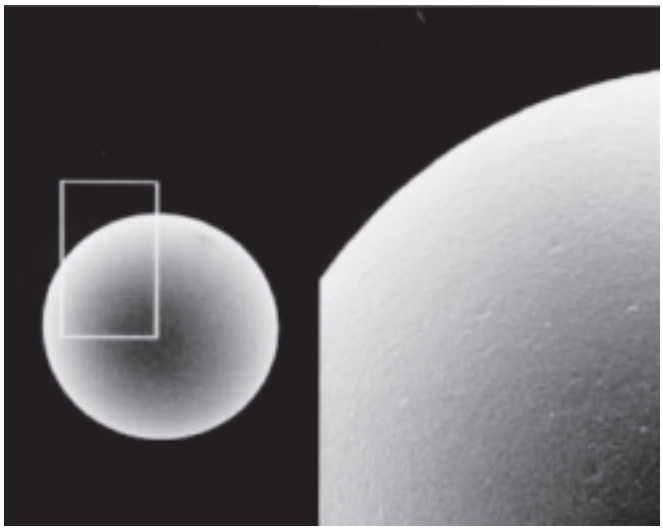
Adsorption Mechanism



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 TiO2 can be reduced by adding acid modifiers to the loading and/or wash buffers.

Bulk Sorbent Materials for Purification & Enrichment of Phosphopeptides

Titansphere™ TiO Bulk Material



While GL Sciences' Phos-TiO spin columns and MonoTip TiO pipette tip based enrichment products are useful for most sample pretreatment applications, some investigators require bulk titanium dioxide media for specialized applications. Titansphere TiO bulk sorbent media is available in 5u and 10u particle sizes in quantities of 500mg.

Purification/Enrichment Protocol

Phos-TiO centrifugation spin columns require only 5 steps:

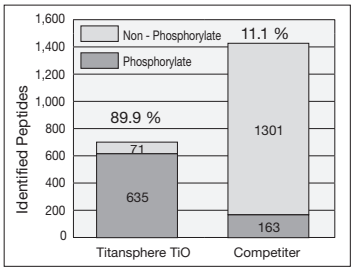
Conditioning → **Equilibrating** → **Adsorption** → **Rinse** → **Elute**

Application

Efficient purification from HeLa Cell Lysate

The data at right shows the superior performance of Titansphere TiO using the HeLa Cell Lysate consisting mainly of non-phosphorylated peptides. Titansphere TiO shows exceptional selectivity - almost 90% of the bound peptides were phosphopeptides, and excellent capacity for total phosphopeptide binding. A competitive TiO product is shown, binding mainly non-phosphorylated peptides and a much lower total number of discreet phosphopeptide species.

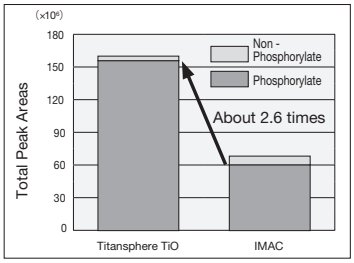
Sample: HeLa Cell Lysate, Sample volume: 50 µg, Titansphere TiO volume: 1 mg



Compare Titansphere TiO with IMAC

The graph at lower right shows how Titansphere TiO compares to an IMAC enrichment using Arabidopsis cell extract. Titansphere TiO provides substantially higher total capacity and a much higher number of discreet phosphopeptides isolated.

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

Identified Numbers of Phosphopeptides

	Phosphorylate	Non - Phosphorylate
Titansphere TiO	846	198
IMAC	474	379

Titansphere™ TiO Part Number

Description		Volume	Cat.No.
Titansphere TiO	5 µm	500 mg	5020-75000
Titansphere TiO	10 µm	500 mg	5020-75010

PHOSPHORYLATION PURIFICATION & ENRICHMENT

Enrichment of Phosphopeptide Using Spin Columns

Titansphere™ Phos-TiO Kit

Titansphere Phos-TiO kits contain titansphere media in a tip-column designed for use with centrifugal solution flow. These spin columns offer the same TiO material provided in bulk form in convenient 200uL (3 mg TiO) and 10uL (1 mg TiO) sizes, and include waste and collection tubes as well as all required buffers.

Features

Easy to Operate

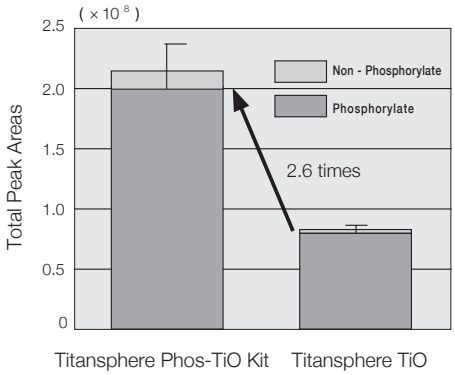
Only 5 steps (completed in about 40 minutes) are required for sample enrichment. Many individual samples can be processed simultaneously without cross contamination.

Phosphopeptide Loading Capacity

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

Performance

Optimal TiO beads are used for Titansphere Phos-TiO Kit.



The existing Titansphere TiO beads were improved for better adsorption capacity of phosphopeptides. Compared to the existing Titansphere beads, 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 beads: 1 mg

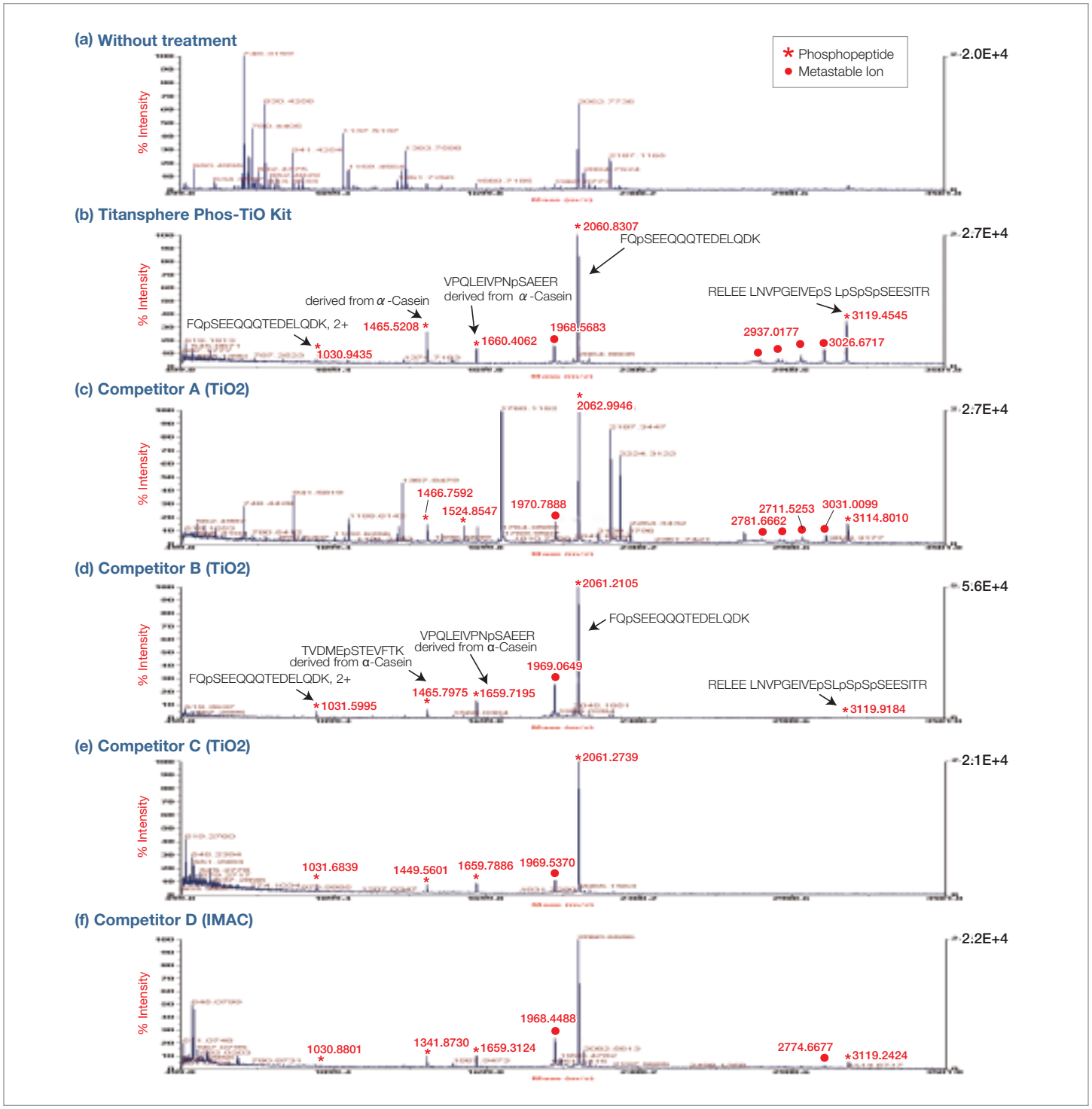
Identified Numbers of Phosphopeptides

	Phosphorylate	Non-Phosphorylate
Titansphere Phos-TiO Kit	996	185
Titansphere TiO	635	71

Titansphere Phos-TiO Kit was developed based on the cooperation from Dr. Yasushi Ishihama from Graduation School of Pharm Sci, Kyoto University.

Application

Fig. 1 - Phos-TiO Kits outperform 4 compeitive TiO based products for phosphopeptide enrichment (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 – e) Titansphere 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.

PHOSPHORYLATION PURIFICATION & ENRICHMENT

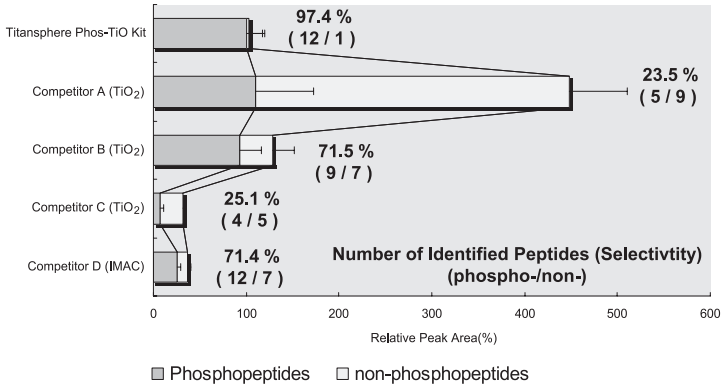
Application

Fig. 2 Comparison Between Titansphere Phos-TiO Kit and 4 Other Market Available Phosphopeptides Enrichment Methods (LC-MS)

Tryptic digest of a-casein, Futein and Phosvitn (each 2.5 µg) were used compare to observe the purification efficiency.

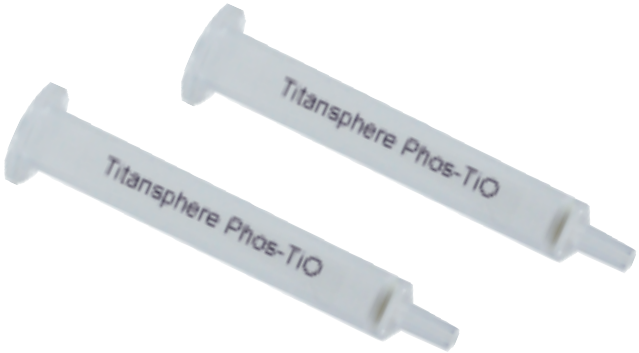
The peak area value of phosphopeptides purified by Titansphere Phos-TiO Kit is shown as 100% (n=3)

The % shown in the Fig. 2 is ratio of phosphopeptides peak area value in the detected peptides peak area value. Also (%) in the Fig. 2 shows the numbers of identified peptides (phosphopeptides/non-phosphopeptides)



Titansphere™ Phos-TiO for Large Volume Samples

GL Sciences now introduces larger versions of these spin columns as an extension of the Phos-TiO product line, including a 3 mL column containing 50 mg of our unique titanium dioxide (TiO2), and another column containing 100 mg of our TiO2.



Titansphere Phos-TiO 50 mg/3 mL and 100 mg/3 mL spin columns

Typical Operating Conditions

Phos-TiO columns 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 column internal configuration and particle size of the TiO beads requires centrifugal elution of all solutions.

Cartridge

Conditioning

Conditioning solution & Rinsing solution
Buffer A：80 % Acetonitrile (0.5 % TFA)
Buffer B：300 mg/mL of lactic acid in Buffer A

Sample loading

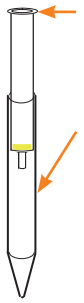
Rinse

Elute

Eluate
5 % ammonium aqueous solution
5 % pyrrolidine aqueous solution

Purified & enriched phosphopeptides
*MonoSpin C18 is recommended for desalting.

Centrifuge



Titansphere Phos - Tio

Centrifuge tube 15 mL

Centrifuge speed 100 - 500 x g

Specifications

Sample	Try (PO_3H_2) - Angiotensin II	
Particle size	10 µm	
Cartridge	50 mg/3 mL	100 mg/3 mL
Binding Capacity	50 µg	100 µg

* The maximum sample loading volume depends on the matrix composition, concentration, freedom from particulates, and viscosity.

Titansphere™ Phos-TiO Kit Part Numbers

Description	Column Size	Quantity	Cat.No.
Titansphere Phos-TiO Kit	1 mg/10 µL	24 times	5010-21309
		96 times	5010-21310
	3 mg/200 µL	24 times	5010-21311
		96 times	5010-21312

Titansphere™ Phos-TiO Column Part Numbers

Description	Volume	Quantity	Cat.No.
Titansphere Phos-TiO Tip	10 µL	24 pcs	5010-21302
		96 pcs	5010-21303
	200 µL	24 pcs	5010-21307
		96 pcs	5010-21308

Description	Column Size	Qty(packed unit)	Cat.No.
Titansphere Phos-TiO	50 mg/3 mL	25 (1 pcs)	5010-21290
	100 mg/3 mL	25 (1 pcs)	5010-21291

Description	Volume	Qty(packed unit)	Cat.No.
Lactic Acid for Titansphere Phos-TiO	15 mL	1 pcs	5010-21295

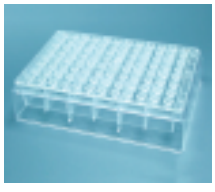
Ordering Information for Reusable Adaptors which are used to mount Phos-TiO spin columns in collection tubes or 96-well plates.

Description	Quantity	Cat.No.
Centrifuge Adapter (10 µL , 200 µLtips)	24 pcs	5010-21514
96well plate centrifuge adapter for 10 µL Tips	1 pcs	5010-21340
	2 pcs	5010-21342
96well plate centrifuge adapter for 200 µL Tips	1 pcs	5010-21341
	2 pcs	5010-21343

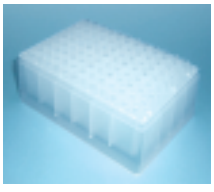
96 well plate adapter is compatible with SBS standard plates.

Centrifuge Adapter

How to Use



96well plate centrifuge adapter for 10 µL Tips



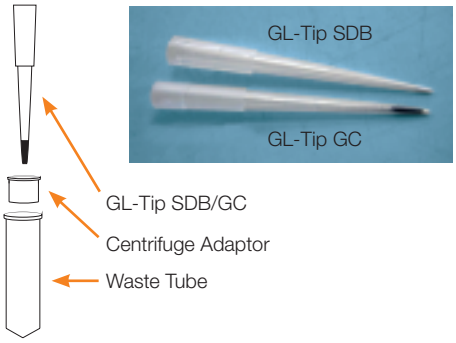
96well plate centrifuge adapter for 200 µL Tips

SAMPLE DESALTING

Desalting of TiO2-Enriched Samples Prior to LC/MS

GL-Tip™ SDB and GL-Tip™ GC

Phosphopeptides isolated using TiO2-based medias are typically desalted prior to analysis by LC/MS, typically using a C18 (hydrophobic) micropipette tip. GL Sciences' SDB (styrene divinylbenzene) and GC (graphite carbon) centrifuge-operated micropipette GL-Tips™ retain more hydrophobic and hydrophilic peptides, respectively, than C18-based tips.



GL-Tip™ SDB and GC Features

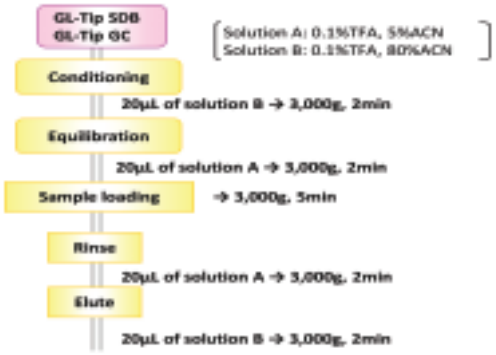
Highly Retentive for Hydrophobic and Hydrophilic Peptides

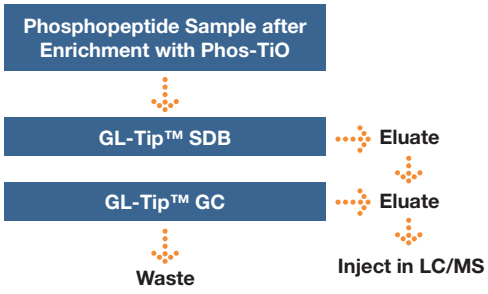
GL-Tip™ SDB are more hydrophobic than C18 medias and allow retention of a wider range of phosophopeptides with high yield, allowing more accurate analysis of phosphopeptide species present in the sample. GL-Tip™ GC retain many more hydrophilic phosphopeptides than does C18; by using a combination of GL-Tip™ SDB and GC, almost all peptide samples can be de-salted without sample losses due to lack of retention.

Easy to operate

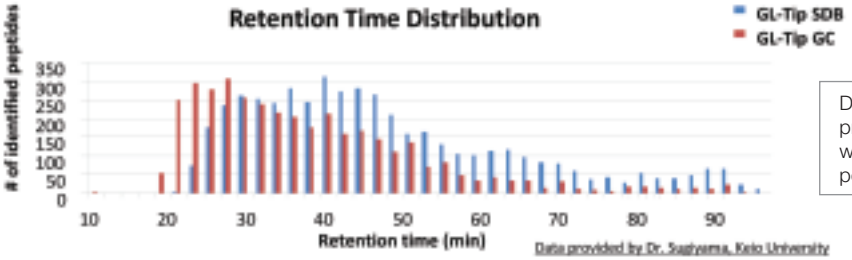
Phosphopeptide-enriched samples are easily loaded, washed, and eluted using the same centrifuge-based technique used with Phos-TiO tips.

Recommended Protocol for GL-Tips™





Application - Compare Relative Retention of Peptides Collected Using GL-Tip™ SDB and GC Desalting Tips



Data indicating that GL-Tip™ SDB preferentially binds hydrophobic peptides while GC preferentially binds hydrophilic peptides.

GL-Tip™ Specifications

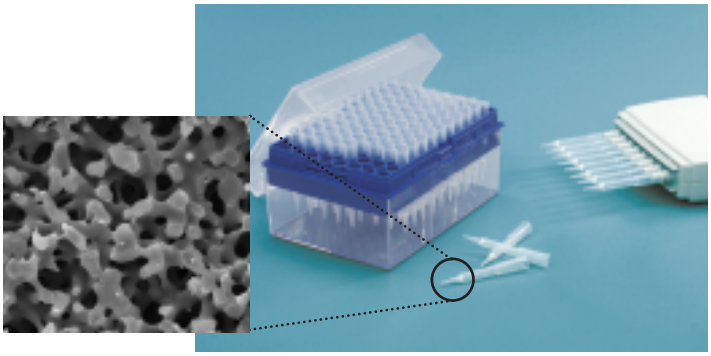
Product	Test Peptide	Tip Volume	Binding Capacity*
GL-Tip™ SDB	Tyr $(PO_3H_2)_4$-Angiotensin II	200 uL	60 ug
GL-Tip™ GC	Gly-Gly-Tyr-Arg	200 uL	30 ug
* Maximum sample loading volume and retained capacity varies with sample matrix			

GL-Tip™ Ordering Information

Description	Quantity	Tip Volume	Order Number
GL-Tip™ SDB	96/pkg	200 uL	7820-11200
GL-Tip™ GC	96/pkg	200 uL	7820-11201
GL-Tip™ Adaptor*	1 ea		5010-21514
*Adaptors are reusable			

Enrichment of Phosphopeptides Using Pipette Method

MonoTip™ TiO



MonoTip TiO pipette-based columns contain GL Sciences' silica monolith completely coated with nanoparticles of titanium dioxide.

MonoTip offers the convenience of rapid enrichment and are still popular today; however, Phos-TiO columns contain an advanced form of TiO2 which provide superior performance and allows many individual samples to be processed simultaneously. For new customers, we recommend evaluating Phos-TiO products as a first choice.

MonoTip Features

Easy to Operate

Protein digests samples are easily loaded, washed, and eluting using a pipette (single or multiple port).

Useful For Relatively Large Samples

MonoTip TiO pipette tips are used for sample volumes of 50 - 200 µL, with approximately 5ug of phosphopeptide bound per tip. Owing to the open prorosity of the monolith contained in these tips, samples can be eluted with small volumes, thereby substantially concentrating phosphopeptides compared with the initial loading volume.

Application

Sample: β-Casein
1 phosphopeptide: FQpSEEQQQTEDELQDK(MW=2061)
4 phosphopeptides: RFLEELNVPGEIVEpSLpSpSpSpSEESLTR(MW=3122)

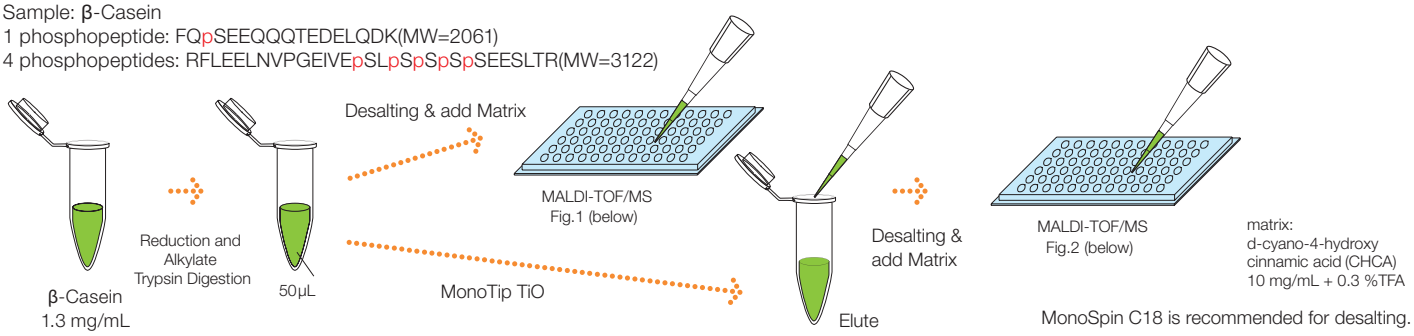
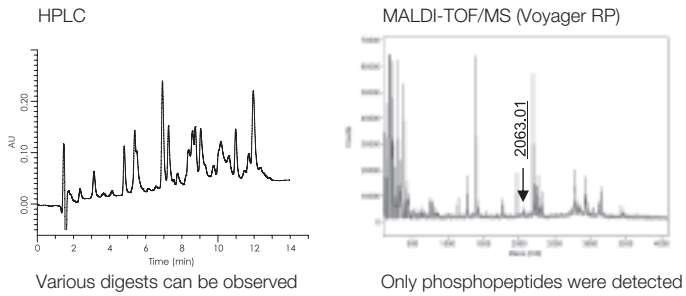


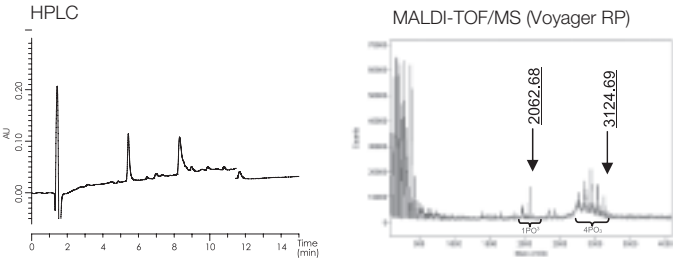
Fig.1: Before purification (Almost no phosphopeptides was enriched)



Various digests can be observed

Only phosphopeptides were detected

Fig.2: After purification (Phosphopeptides were selectively enriched)



Only 2 digests can be observed when using MonoTip TiO

Phosphopeptides and 4 phosphopeptides are clearly detected

Specifications

Parameter	MonoTip TiO
Operation time	Approx. 6 mins
Suitable sample volume	20 ~ 200 µL
Sample loading volume	up to 5 µg
Tip volume	200 µL
Packing material	Silica Monolith (Highly pure silica gel)
Through-pore Diameter	10 ~ 20 µm
Meso-pore Diameter	200 Å (20 nm)
Surface area	200 m2/g
Functional group	Dioxide titan coating

MonoTip™ TiO Part Numbers

Description	Volume	Quantity	Cat.No.
MonoTip TiO	200 µL	24 pcs	5010-21007
		96 pcs	5010-21005

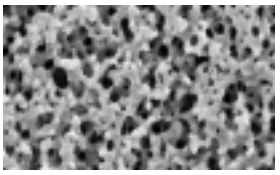
"Based on monolithic technology, Merck KGaA, Darmstadt, Germany"

LOW-MOLECULAR COMPOUNDS EXTRACTION & PURIFICATION

Monolithic SPE Column for the Purification and Enrichment of Small Amount Sample

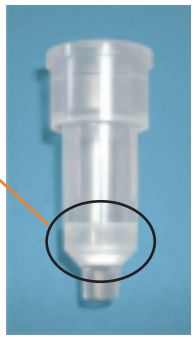
MonoSpin™ Series

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.



Silica monolith Enlarged picture

Centrifuge Operation



MonoSpin

Features

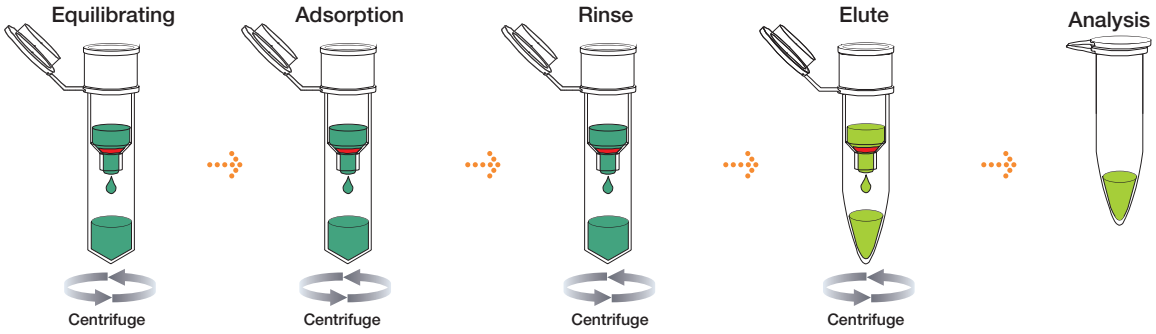
Easy to Operate
Centrifuge elution allows loss-free and efficient processing of many samples simultaneously, with little or no liquid retained by the separation matrix.

Fast
Excellent mass transfer and rapid sample binding on MonoSpin's monolith silica allows extremely rapid sample preparation compared with other methods.

Ideal for Small Sample Volumes
Excellent for the pretreatment for samples of 50-800 µL

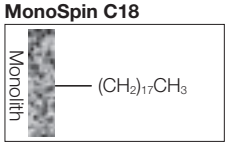
Wide Variety of Functional Groups
Eight surface chemistries are currently availble in our MonoSpin format, allowing almost any type of compounds to be purified.

How to Operate



Product Lineup

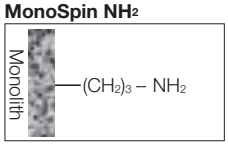
MonoSpin C18



Octadecyl functional group.

Optimal for drug extraction in biological samples, and desalting & enrichment of peptide samples.

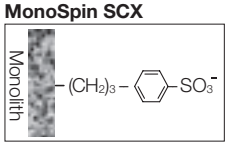
MonoSpin NH₂



Bonded with aminopropyl.

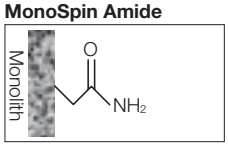
Optimal for the enrichment of sugar chain and/or hydrophilic compounds by HILIC mode.

MonoSpin SCX



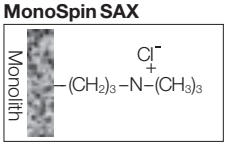
Bonded with propyl benzene sulfonic acid combining both strong cation exchange & hydrophobic interaction. Optimal for the extraction of basic drugs.

MonoSpin Amide



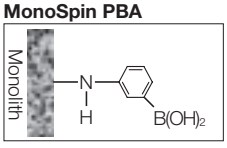
Bonded with amide group. Optimal for the extraction of sugar chains and various acidic and basic hydrophilic compounds by HILIC mode.

MonoSpin SAX



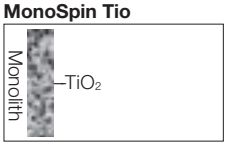
Bonded with Trimethyl aminopropyl combining both strong anion exchange & weak hydrophobic interaction. Optimal for the extraction of acidic drugs.

MonoSpin PBA



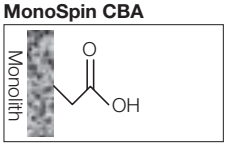
Specific column combined with phenyl boric acid. Excellent for the selective extraction of cis diol compounds, such as catechol amines.

MonoSpin Tio



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

MonoSpin CBA



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

Application

Drugs in Urine (MonoSpin C18)

MonoSpin C18 has been used to rapidly enrich and concentrate drug compounds and their metabolites from urine, as detailed below

Solvents
A (Rinsing) : CH_3CN/Alkaline buffer (pH 12) = 10/90
B (Eluent) : CH_3CN/0.1 % H_3PO_4 (pH 3) 20 mM IPCC = 25/75
(IPCC-08 : Sodium 1-Octane sulfonate)

All the centrifugal processes at 3,000 rpm

Sample: 500 µL
Add alkali buffer (pH 12) 400 µL
Add I.S. Methoxyphenamine 20 µL

Conditioning
1. Set the waste fluid tube and add 500 µL of acetonitrile, then centrifuge (1 min)
2. Add 500 µL of boiled water and centrifuge (1 min)
3. Abandon the waste fluid

MonoSpin C18 Sample loading

Put all the sample and centrifuge for 5 mins Abandon the waste fluid

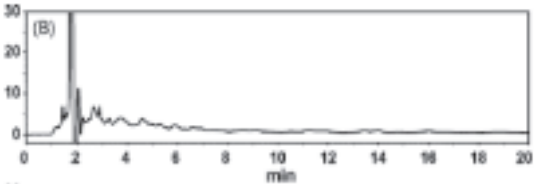
Rinse

Add 500 µL of A and centrifuge for 1 min Abandon the waste fluid and set the recovery tubes

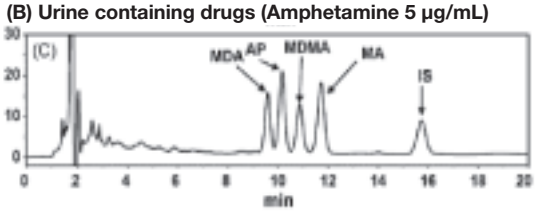
Elute

Add 200 µL of B and centrifuge for 1 min to recover the eluent solution

(A) Urine without drugs



(B) Urine containing drugs (Amphetamine 5 µg/mL)



Conditions

Column:	C18 Column (150 × 4.6 mm I.D.)
Eluent:	CH_3CN/0.1 % H_3PO_4, 20 mM IPCC-08 =25/75
Flow Rate:	1mL/min
Detection:	UV 215 nm (PDA Detector)
Injection Vol:	10 µL
Analyte:	Methamphetamine (MA) Amphetamine (AP) 3,4-Methylenedioxymethamphetamine (MDMA) 3,4-methylenedioxyamphetamine (MDA)

<*J. Chromatogr A* 1208 (2008) 71-75>

Reference

Extraction of amphetamines and methylenedioxyamphetamines from urine using a monolithic silica disk-packed spin column and high-performance liquid chromatography-diode array detection
J Chromatogr A. 2008 Oct 24;1208(1-2): 71-5

Simultaneous determination of dibucaine and naphazoline in human serum by monolithic silica spin column extraction and liquid chromatography-mass spectrometry.
J Chromatogr B Analyt Technol Biomed Life Sci. 2008 Sep 1; 872(1-2): 186-90

Simultaneous determination of amitraz and its metabolite in human serum by monolithic silica spin column extraction and liquid chromatography-mass spectrometry.
J Chromatogr B Analyt Technol Biomed Life Sci. 2008 May 1;867(1): 99-104.

Purification of pyridylaminated saccharides (MonoSpin NH2)

MonoSpin NH2 shows ~100% recovery of the pyridylaminated saccharides from original impure sample.

Solvents
A (Coupling liquid, rinsing) : CH_3CN : H_2O : HCOOH=90 : 10 : 0.1
B (Eluent) : CH_3CN : H_2O : HCOOH=50 : 50 : 0.1
Formic acid, acetic acid and TFA can be added

All the centrifugal processes at 5,000 rpm

Dissolve the sample so the concentration of acetonitrile becomes 90-95%

Conditioning
1. Set the waste fluid tube and add 500µL of B then centrifuge (2 mins)
2. Add 500µL of A and centrifuge (2 mins) and abandon the waste fluid
3. Abandon the waste fluid

MonoSpin NH2 Sample loading

Put all the sample and centrifuge for 2 mins Abandon the waste fluid

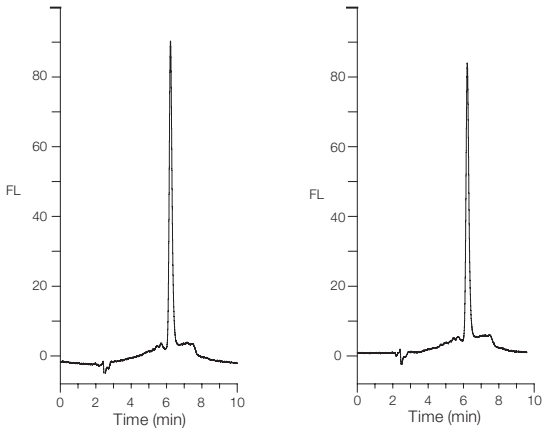
Rinse

Add 500µL of A and centrifuge for 2 mins Abandon the waste fluid and set the recovery tubes

Elute

Add 50 - 800 µL of B and centrifuge for 2 mins to recover the eluent solution

Before Purify **After Purify**



Conditions

Column:	NH_2 Column (250 × 4.6 mm I.D.)
Eluent (A):	200 mM Acetic acid-Triethylamine (pH 7.3) / CH_3CN=35/65
(B):	200 mM Acetic acid-Triethylamine (pH 7.3) / CH_3CN=50/50
	A/B=80/20-10 min-40/60
Flow Rate:	1 mL/min
Detection:	FL Ex 310 nm Em 380 nm
Injection Vol:	5 µL
Analyte:	PA001 (TaKaRa BIO) 1 pmol/500 µL

LOW-MOLECULAR COMPOUNDS EXTRACTION & PURIFICATION

Recovery of basic drugs in serum (MonoSpin SCX)

Recovery of basic drugs in serum.

Solvents
A (Coupling liquid): 20 mM of potassium phosphate buffer (pH 7.0)
B (Eluent): 5 % concentration ammonium water - methanol
C : A + drug: Propranolol hydrocholoride (3.25 µL/mL)

All the centrifugal processes at 13,000 rpm

Sample : Serum100µL
+ Add 200µL of C

Conditioning
Set the waste fluid tube
Add 400 µL of A and centrifuge (1 min)

MonoSpin SCX Sample loading

1. Put all the sample and centrifuge for 1 min
2. Abandon the waste fluid

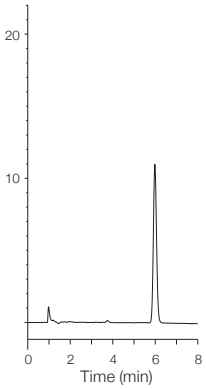
Rinse

1. Add 300µL of A and centrifuge for 1 min
2. Abandon the waste fluid and set the recovery tubes

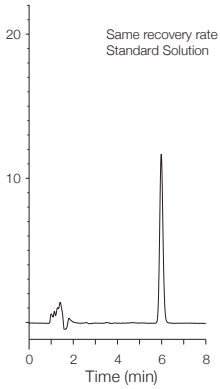
Elute

Add 300µL of B and centrifuge
for 1 min to recover the eluent solution

Recovery from Standard Solution



Recovery from Serum



Conditions

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

Catecholamines in Urine (MonoSpin PBA)

Cis-diol compounds and catecholamines can be purified & enriched by MonoSpin PBA.

Solvents
A (Rinsing): 100 mM HEPES - NaOH (pH 8.5)
B (Coupling liquid): 1.5 M HEPES - NaOH (pH 8.5)
C (Eluent): 1 % acetic acid

All the centrifugal processes at 10,000 rpm

Sample: 450 µL Add 50 µL of B
3,4-Dihydroxybenzylamine(I.S.) 10 µL

Conditioning
1. Set waste fluid tubes, put 500 µL of C and centrifuge for 1 min
2. Put 500 µL of A and centrifuge for 1 min
3. Abandon the waste fluid

MonoSpin PBA Sample loading

1. Put all the sample and centrifuge for 1 min
2. Abandon the waste fluid

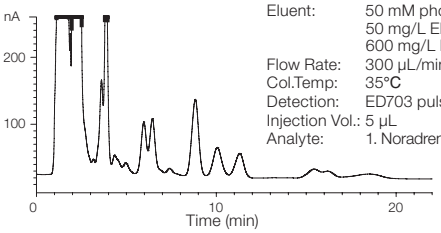
Rinse

1. Add 500µL of A and centrifuge for 1 min
2. Abandon the waste fluid and set the recovery tubes

Elute

Add 50 - 500 µL of C and centrifuge
for 1 min to recover the eluent solution

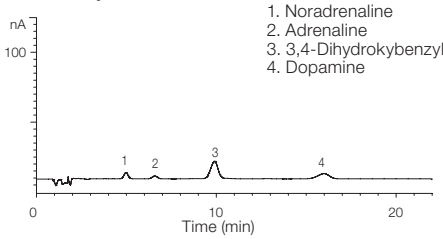
Umpurified



Conditions

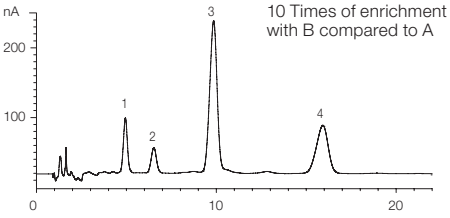
Column:	Inertsil ODS-3 (150 × 2.1 mm I.D.)
Eluent:	50 mM phosphate Buffer (pH5.6) 50 mg/L EDTA 600 mg/L IPCC-08/CH_3OH=90/10
Flow Rate:	300 µL/min
Col.Temp:	35°C
Detection:	ED703 pulse diamond electrode +800 mV Ag/AgCl
Injection Vol.:	5 µL
Analyte:	1. Noradrenaline, 2. Adrenaline, 3. DHBA, 4. Dopamine

Purify with MonoSpin PBA
Elute 500 µL..... A



1. Noradrenaline
2. Adrenaline
3. 3,4-Dihydrokybenzylamine
4. Dopamine

Purify with MonoSpin PBA
Elute 50 µL..... B



10 Times of enrichment effect
with B compared to A

Phosphoric Amino Herbicides in Urine (MonoSpin TiO)

Phosphoric amino herbicides can be selectively purified and enriched from of complex matrices such as urine.

Solvents
A (Rinsing): 0.1% TFA in 80 % CH_3CN aqueous solution
B (Rinsing): 0.1% TFA in 50 % CH_3CN aqueous solution
C (Eluent): 2 % NH2 aqueous solution

All the centrifugal processes at 5,200 × g

Sample : 10µL and I.S.
Dilute with 40µL of H_2O
Add 100µL of B and mix

Conditioning
1. Set waste fluid tubes, put 20µL of A and centrifuge for 2 mins
2. Put 20µL of B and centrifuge for 2 mins

MonoSpin TiO Sample loading

1. Put all the sample and centrifuge for 10 mins
2. Re-put the solution came through the column into the column
3. Centrifuge for 10 mins and abandon the waste fluid

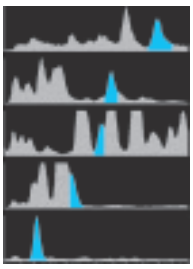
Rinse

1. Add 20µL of B and centrifuge for 2 mins
2. Add 20µL of A and centrifuge for 2 mins
3. Abandon the waste fluid and set the recovery tubes

Elute

Add 20µL of C and centrifuge for 5 mins to recover the eluent solution

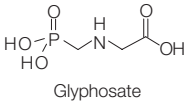
No pretreatment **Pretreatment by MonoSpin TiO**



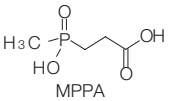
Bialaphos
Glyphosate
MPPA
Glufosinate
AMPA

Conditions

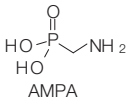
Column: C18 Column (150 × 2.1 mm I.D.)
Eluent: CH_3OH/20 mM HCO_2NH_4 (pH3.0) = 15/85
Flow Rate: 200 µL/min
Detection: SIM
Injection Vol.: 5 µL
Analyte: Bialaphos, Glyphosate, MPPA, Glufosinate, AMPA (1ppm each)



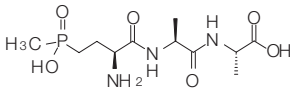
Glyphosate



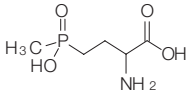
MPPA



AMPA



Bialaphos



Glufosinate

Specifications

Description	MonoSpin
Packing material	Silica Monolith (Highly pure silica gel)
Through - pore Diameter	5 µm
Meso-pore Diameter	100 Å (10 nm)
Surface Area	350 m^2/g
Sample Volume	50 - 800 µL

MonoSpin™ Part Number

Description	Quantity	Cat.No.
MonoSpin C18	50 pcs	5010-21700
	100 pcs	5010-21701
MonoSpin Amide	50 pcs	5010-21727
	100 pcs	5010-21728
MonoSpin CBA	50 pcs	5010-21729
	100 pcs	5010-21730
MonoSpin NH2	50 pcs	5010-21710
	100 pcs	5010-21711
MonoSpin SCX	50 pcs	5010-21725
	100 pcs	5010-21726
MonoSpin SAX	50 pcs	5010-21720
	100 pcs	5010-21721
MonoSpin PBA	50 pcs	5010-21715
	100 pcs	5010-21716
MonoSpin TiO	50 pcs	5010-21705
	100 pcs	5010-21706

"Based on monolithic technology, Merck KGaA, Darmstadt, Germany"

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http://www.glsciences.com/products.html

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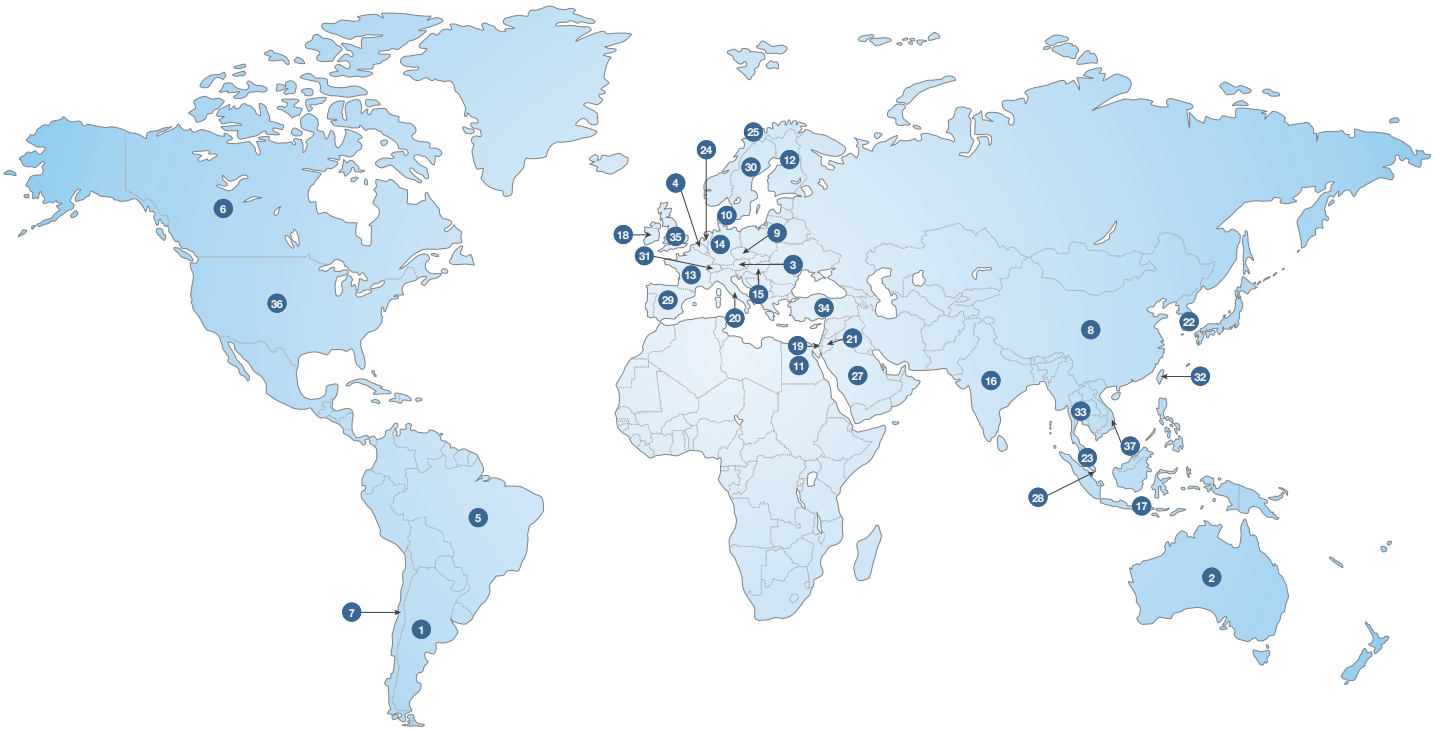

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15 HUNGARY
16 INDIA
17 INDONESIA
18 IRELAND
19 ISRAEL
20 ITALY
21 JORDAN
22 KOREA
23 MALAYSIA
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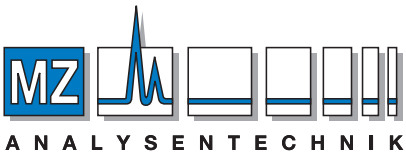

AUTHORIZED DISTRIBUTOR

MZ-Analysentechnik GmbH
Barcelona-Allee 17 • D-55129 Mainz
Tel +49 6131 880 96-0
Fax +49 6131 880 96-20
e-mail: info@mz-at.de
www.mz-at.de

GL Sciences Inc.
22-1 Nishishinjuku 6-chome, Shinjuku-ku
Tokyo 163-1130
Japan
Tel: +81.3.5323.6620
E-mail: world@gls.co.jp

GL Sciences B.V.
De Sleutel 9
5652AS Eindhoven
The Netherlands
Tel: +31.40.2549531
E-mail: info@glsciences.eu

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