



HICHROM

Chromatography Columns and Supplies

SAMPLE PREPARATION Pipette & Syringe Tips

Catalogue 9

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PIPETTE TIPS

- Simple, fast sample preparation with minimal sample loss
- No contamination from supporting matrix
- Sample volumes as small as 0.1 μl

A range of micropipette tips is manufactured by Glygen Corporation for the preparation and separation of biological samples for HPLC, MALDI, CE, desalting and electrophoresis. In addition to non-bonded silica and reversed-phase materials, NuTips and TopTips are available packed with PolyLC ion-exchange materials (see pages 196-199) or with titania, zirconia, graphitic carbon and affinity media.

NuTip™

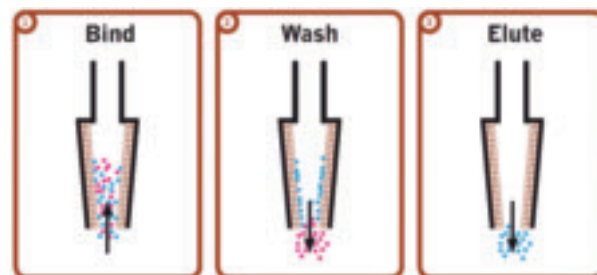
NuTip™ enables purification of low concentration or low volume samples by maximising the surface area in contact with the sample. The chromatographic media is directly attached to the inner surface of the pipette tip without using polymers or glue. This helps avoid potential problems with contamination or permeability. Figure 1 shows the basic operating principles of purification using NuTips.

Specifications

Tip Volume (μl)	Sample Volume (μl)	Binding Capacity (μg)	Amount Chromatographic Material (mg)
1 - 10	0.5 - 10	1	30
10 - 200	2 - 25	2.5	75
20 - 200	5 - 50	15	400

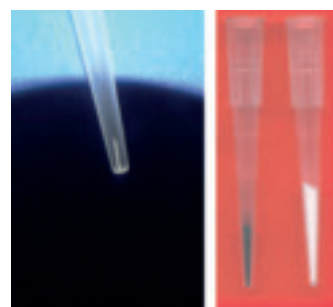


NuTip



Unpurified sample drawn into NuTip. Target molecule binds. Impurities expelled. Target molecules remain bound. Solvent releases bound target molecules. Purified sample collected.

Figure 1. Basic operating principles of NuTip



TopTip

TopTip™

TopTip™ is a pipette tip with a fine slit (1-2 μm) at the bottom which enables liquid to pass through but retains chromatographic material (20-30 μm) in the tip. This eliminates the need for a filter, thereby reducing dead volume, loss of sample and contamination risk. Pressure can be applied via centrifuge, pipette, syringe or vacuum manifold. Figure 2 shows the basic operating principles of purification using TopTips.

Specifications

Tip Volume (μl)	Sample Volume (μl)	Binding Capacity (μg)	Amount Chromatographic Material (mg)
1 - 10	1 - 10	400	4
10 - 200	2 - 25	1000	10
100 - 1000	20 - 1000	5000	50



Sample top-loaded. Target molecule binds to media. Pressure forces impurities out of slit. Target bound. Target molecules released from media. Purified sample collected.

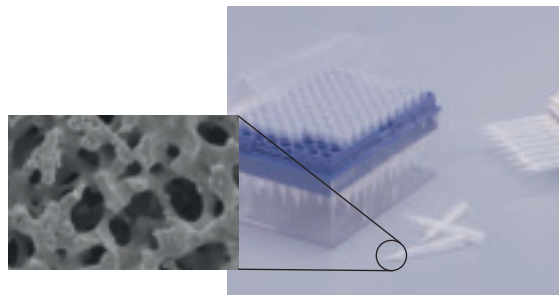
Figure 2. Basic operating principles of TopTip

Both NuTip and TopTip products are available packed with a range of materials to suit a wide range of applications. Please enquire for further details.

MONOTIP™

- MonoTip C18 for desalting and enrichment of peptides and proteins
- MonoTip Trypsin for fast trypsin digestion
- MonoTip TiO for phosphopeptide enrichment

MonoTip™ pipette tips, manufactured by GL Sciences, are designed for the purification and enrichment of femtomole to micromole levels of peptides and proteins prior to MALDI-MS and LC-MS analyses. The monolithic silica consists of a double pore structure comprising a network of throughpores and smaller mesopores. This unique monolithic structure contributes to low pressure-drop and strong analyte interactions.

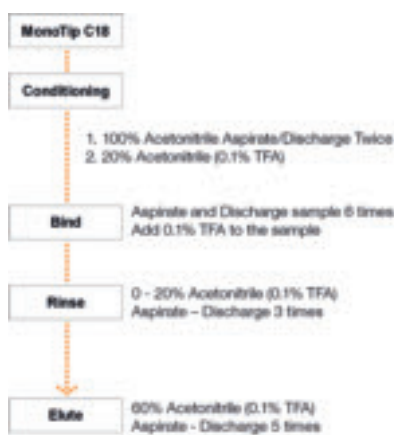


MonoTip Specifications

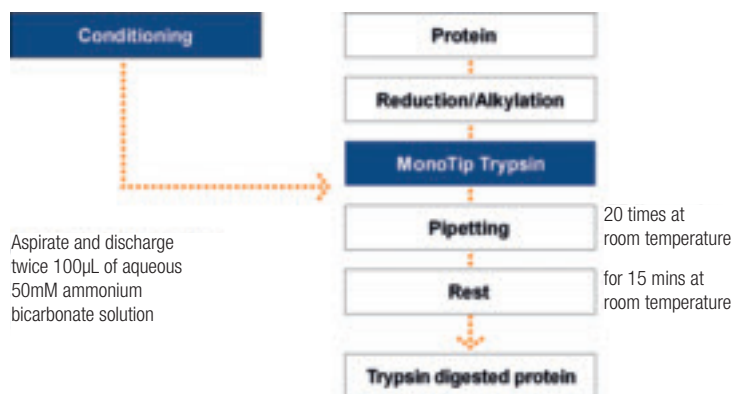
Description	MonoTip C18	MonoTip Trypsin	MonoTip TiO
Tip volume (µl)	200	200	200
Bonded phase	Octadecyl	TPCK treated trypsin	Titanium dioxide coating
Specific surface area (m ² /g)	200	100	200
Throughpore diameter (µm)	10 - 20	10 - 20	10 - 20
Mesopore diameter (Å)	200	300	200
Sample capacity	100µg (angiotensin II)	100µg (denatured BSA) 2.5 BAEE unit/tip	5µg
Sample volume (µl)	20 - 200	20 - 200	50 - 200

MonoTip C18 – An easy pipetting operation enables the enrichment and demineralisation of proteins and peptides. Salts, detergents and other hydrophilic contaminants pass through the tip unretained, whereas target peptides and proteins have a strong affinity for the hydrophobic monolithic silica surface, and can then be recovered in a concentrated and purified form. MonoTip C18 is efficient for peptide and protein samples in the pmol to nmol range with molecular weight up to 40kDa.

MonoTip Trypsin – MonoTip Trypsin pipette tips are designed for the digestion of proteins in proteomic analyses. They consist of TPCK treated bovine pancreas immobilised on monolithic silica, which catalyses the quick digestion of reduced and alkylated proteins at room temperature with just a few simple operations. At 37°C, it usually takes more than 10 hours to digest trypsin. With MonoTip Trypsin, it takes only a few minutes pipetting and an overall operation time of 20 minutes at room temperature.



Operation of MonoTip C18



Operation of MonoTip Trypsin

MonoTip TiO – MonoTip TiO pipette tips contain GL Sciences' silica monolith completely coated with nanoparticles of titanium dioxide (TiO₂). MonoTip TiO pipette tips are used for sample volumes of 50 - 200µl, with approximately 5µg of phosphopeptide bound per tip. Due to the open porosity on the monolith contained in these tips, samples can be eluted with small volumes, thereby substantially concentrating phosphopeptides when compared to the initial loading volume. Please see page 311 for details of the more advanced Phos-TiO kits.

Ordering Information

Description	Pack Size		
	8	24	96
MonoTip C18	5010-21001	-	5010-21000
MonoTip Trypsin	5010-21011	5010-21012	5010-21010
MonoTip TiO ¹	5010-21006	5010-21007	5010-21005

¹ Please note that Phos-TiO columns contain an advanced form of TiO₂ which provides superior performance and allows many individual samples to be processed simultaneously. For new methods, it is recommended to use Phos-TiO products as first choice (see page 311).

PURIFICATION AND ENRICHMENT OF PHOSHOPEPTIDES

Enrichment of phosphopeptides and the relative reduction of non-phosphorylated peptides are critical for accurate analysis of protein digests by MALDI-MS and LC-MS analyses. GL Sciences' TiO₂ (titania) products have proved to be particularly effective in this role, showing increased selectivity compared with traditional IMAC technologies. Figure 1 shows a 2.6 times increase in phosphopeptide peak area from an Arabidopsis extract with Titansphere™ TiO compared to IMAC.

Phosphate groups are preferentially adsorbed to the surface of titanium dioxide under acidic conditions and are eluted under basic conditions. Non-phosphorylated acid peptides non-specifically bound to the TiO₂ can be reduced by adding acid modifiers to the loading and/or washing buffers.

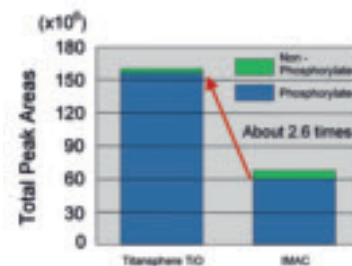
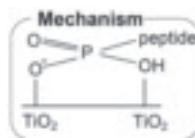


Figure 1. Comparison with IMAC

*The comparative data presented here may not be representative for all applications.

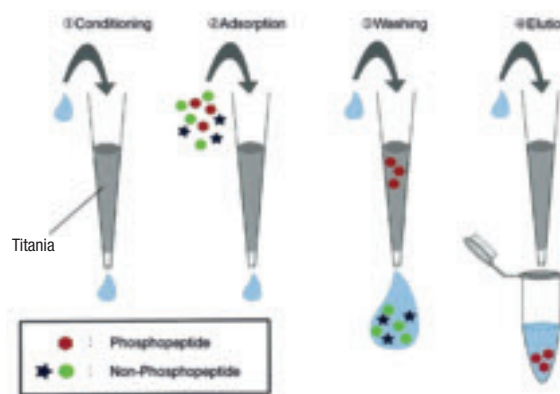
Titansphere™ Phos-TiO Kit

Titansphere Phos-TiO kits contain Titansphere media in a tip-column designed for use with centrifugal solution flow. These spin columns offer TiO₂ material in convenient 200µl (3mg TiO₂) and 10µl (1mg TiO₂) sizes, and include waste and collection tubes. These TiO₂ beads have been improved compared with the initial Titansphere beads (see page 118) with regard to adsorption capacity of phosphopeptides, and therefore show superior performance to the MonoTip TiO pipette tips.

Larger volume versions of these spin columns are now available, as an extension of the Phos-TiO product line, including a 3ml column containing 50mg of TiO₂ and another 3ml column containing 100mg TiO₂.

Features

- **Easy to operate** – the operation is only 4 steps and the total operation time is only 40 minutes
- **High selectivity** – an enhancer is added to reduce the non-specific adsorption and selectively purify and enrich the phosphopeptide
- **High throughput** – multiple samples can be analysed using 96 well plates
- **High capacity** – the surface activity of the Titansphere particles is optimised for the purification and enrichment of phosphopeptides



Each Titansphere Phos-TiO kit contains spin tips, waste fluid tubes, recovery tubes and instruction manual. A reusable centrifuge adaptor is required for use in combination with each tip or 96 well plate (see below).

Titansphere Phos-TiO Kits

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

Titansphere Columns

Description	Volume	Quantity/pack	Cat. No.	Price
Titansphere Phos-TiO Tip	10µl	24	5010-21302	
		96	5010-21303	
	200µl	24	5010-21307	
		96	5010-21308	

Description	Column Size	Quantity/pack	Cat. No.	Price
Titansphere Phos-TiO	50mg/3ml	25	5010-21290	
	100mg/3ml	25	5010-21291	

Centrifuge Adaptors

Description	Quantity/pack	Cat. No.	Price
Centrifuge adaptor	24	5010-21514	
96WP centrifuge adaptor for 10µl tips	1	5010-21340	
	2	5010-21342	
96WP centrifuge adaptor for 200µl tips	1	5010-21341	
	2	5010-21343	

Please see page 118 for details of Titansphere (500mg) bulk material.



Contents of Phos-TiO kit



Titansphere Phos-TiO tip



Titansphere Phos-TiO



Centrifuge adaptor

GL-TIP™ SDB AND GL-TIP™ GC

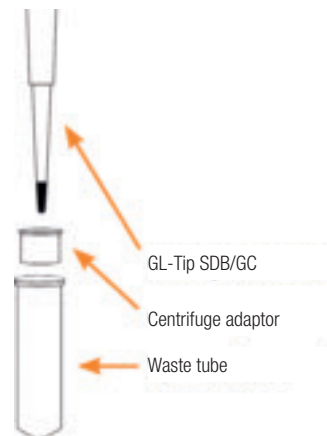
- Effective desalting and enrichment of peptides
- High yield
- Easy to operate

In proteome analysis, it is necessary to desalt and enrich peptides before introducing samples into the MS. GL Sciences' SDB (styrene-divinylbenzene copolymer) and GC (graphite carbon) centrifuge-operated micropipette GL-Tips retain more hydrophobic and hydrophilic peptides respectively than C18-based tips. The strong retentivity of GL-Tip SDB enables more peptides or proteins to be captured than trapped by existing C18 tips. By using a combination of GL-Tip SDB and GC, almost all peptide samples can be desalted.

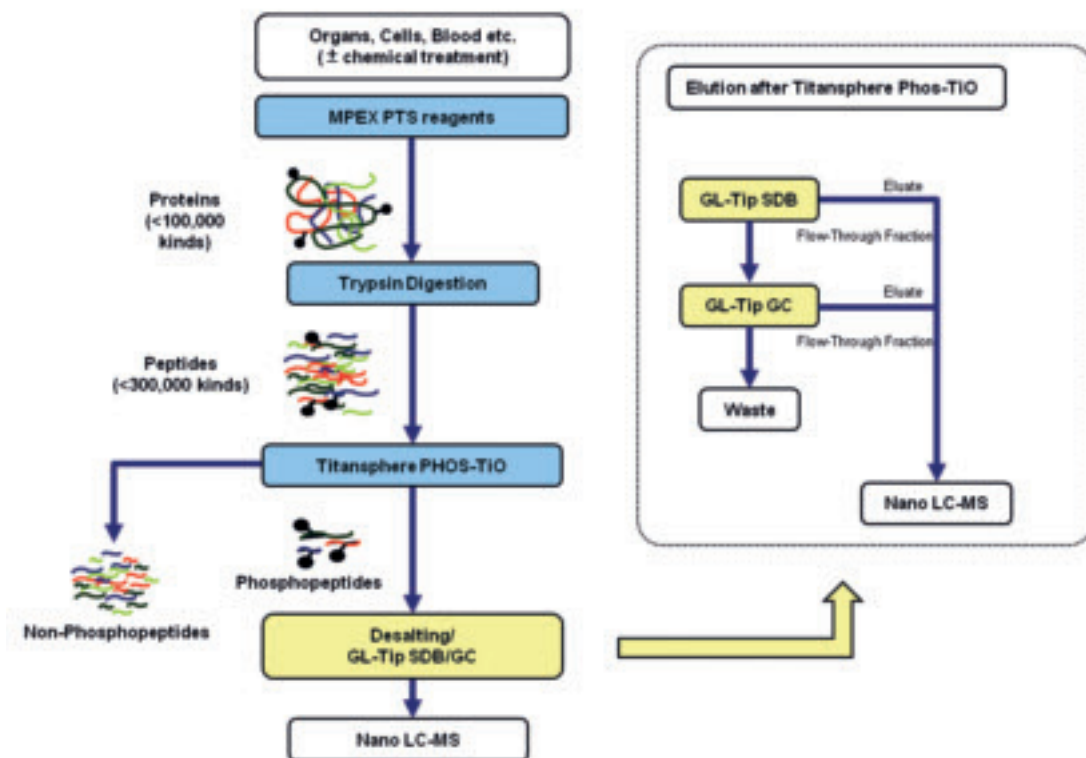
Specifications of GL-Tips

Product	Tip Volume (µl)	Binding Capacity (µg)	Test Peptide
GL-Tip SDB	200	60	Tyr(PO ₃ H ₂)-Angiotensin II
GL-Tip GC	200	30	Gly-Gly-Tyr-Arg

Phosphopeptide-enriched samples are easily loaded, washed and eluted using the same centrifuge-based technique that is used with Phos-TiO (see page 311). The schematic below indicates the overall procedure for tryptic digestion, phosphopeptide enrichment and further fraction desalting prior to nano LC-MS.



Overview



Phosphopeptide enrichment using GL-Tip and Titansphere Phos-TiO

Ordering Information

Product	Quantity	Cat. No.	Price
GL-Tip SDB	96/pk	7820-11200	
GL-Tip GC	96/pk	7820-11201	
GL-Tip adaptor	24/pk	5010-21514	