

# Separation techniques (Proteins)

## Sample Preparation

Sample preparation is arguably the most critical step within the analytical process. Interchim has placed considerable time and energy into its product development programmes to assist users with this aspect of the analysis process. Thanks to these efforts, considerable improvements have been made over the last five years.

**As a result : our very new and revolutionary UptiTip™ (Refer page B.84)**



UptiTip packed

Studies have reported that sample preparation can represent up to 60% of a laboratory technician timetable and is also one of the principal source of error. Sample preparation undoubtedly has a fundamental impact on the subsequent quality of analysis affecting a wide range of analytical parameters.

A variety of proven techniques, such as filtration, dialysis, liquid/liquid extraction and Solid Phase Extraction (SPE), are routinely adopted in today's analytical laboratories to resolve the vast array of sample preparation demands.

SPE has established itself as one of the most popular and flexible tool within the analytical laboratory. It provides effective and efficient sample concentration, it allows purification prior to HPLC, LC/MS, GC or GC/MS (electrophoresis, immuno- or cell-assays) and adaptation to automated techniques such as combinatory chemistry.

## Sample Preparation Extraction

Interchim's laboratories have developed a range of SPE products from their core analytical supports i.e. Silica (Uptiprep) and Polystyrene divinylbenzene (Atoll). The resultant SPE product range provides unparalleled performances and satisfies the customers request for Recovery, Reliability and Reproducibility.

### General Methodology

SPE methods consist of several steps :

#### ◆ Packing

Pack the absorbent with an organic solvent such as MeOH or a solvent mix to eliminate contaminants and activate the media. The absorbent should not be allowed to dry before adding the sample.

#### ◆ Sample introduction

Into the top part of the absorbent bed and either push or pull the sample through the bed at a low flow rate.

#### ◆ Wash

(Only necessary if your compounds of interest has been absorbed onto the bed). Rinse to remove all interfering agents with a solvent or suitable solvent mixture (0 % affinity for the compound of interest, 100 % for interfering agent). A number of washing phases with different solvents may be required. This being usually dependent upon the nature of interfering substances.

#### ◆ Elution

The absorbed sample must be eluted with a solvent or a solvent mixture that has 100 % affinity for the sample and 0 % affinity for any potential interfering agents.

#### ◆ Dry

(If necessary) elute with anhydrous sodium sulfate to remove all traces of water.

#### ◆ Concentration

(If necessary) concentrate the compound of interest by an evaporating step and retake it with the analysis mobile phase before injection for analysis.

Average capacity of the absorbent mass

Silice : 5 to 10 %

Polymeric : > 30 %



UptiTip Coated

Washing volumes are twice the absorbent volume. Chosen absorbents must have excellent affinities for compounds of interest and no affinity for interfering agents. Solvents chosen for the Washing and Elution steps should provide optimal efficiency with the lowest volume.

Low sample recoveries are often due to the selection of an inappropriate bed weight. Excessive bed weights lead to incomplete elution or sample dilution. Reduced bed weights lead to incomplete sample retention.

### Containers

Interchim offer a wide selection of SPE container formats.

- ◆ **Tips**  
Available volumes : 0.1-10 µl ; 10-200 µl  
Frits : no frits
- ◆ **Polypropylene tanks (syringe type) - medical grade**  
Available volumes : 1-3-6-15-25-75-150 ml  
Frits : 10-20-70 µm/PE/PTFE
- ◆ **Large polypropylene tanks (LRC)**  
Volume : 15 ml  
Frits : 20 µm PE
- ◆ **Glass tanks**  
Available volumes : 1-3-6-20 ml  
Frits : 20 µm PTFE-glass fiber
- ◆ **Polypropylene cartridge-medical grade**  
Absorbent mass : 300-600-900 mg  
Frits : 20 µm PE
- ◆ **96 wells plates**  
For development :  
96 well square plate 2 ml with custom filling  
For routine :  
500 µl round well/10 µm PE frits  
2 ml square well/20 µm PE frits  
[Precision bed weight ± 1 %]  
Custom manufacture inquiries are welcome.

### Selection guide

#### Classical SPE - Adsorbant selection guide

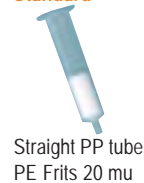
Designation	Nature	Type	Comment	End-capping	Pore size A	Area m <sup>2</sup> /g	Size µm	Shape	Purity
<b>Atoll</b>									
Atoll	PSDVB	ATL	hydrophobic	no	70	800	100	spherical	pure
Atoll	PSDVB	ATH	hydrophilic	no	70	800	75	spherical	pure
Atoll	PSDVB	30XC	high capacity	no	n.c.	1500	30	spherical	pure
Atoll	PSDVB	XC	high capacity	no	n.c.	1500	70	spherical	pure
Atoll	PSDVB	XWP	high capacity	no	wide pore	1200	90	spherical	pure
<b>Biopolymer</b>									
Biopolymer	PSDVB	SPCE	sulfopropyl	no	600	n.c.	30	spherical	pure
Biopolymer	PSDVB	WPCE	carboxymethyl	no	600	n.c.	30	spherical	pure
Biopolymer	PSDVB	WPAE	DEAE	no	600	n.c.	30	spherical	pure
Biopolymer	PSDVB	SPAЕ	QA	no	600	n.c.	30	spherical	pure
<b>Upti-prep</b>									
Upti-prep	Silica	C18-S	% C : 18	yes	60	500	50	spherical	pure
Upti-prep	Silica	C18U	% C : 16	no	60	500	50	spherical	pure
Upti-prep	Silica	C18-S2F	high flow	yes	60	500	140	spherical	pure
Upti-prep	Silica	C18U-S2F	high flow	no	60	500	140	spherical	pure
Upti-prep	Silica	C8-S	% C : 11	yes	60	500	50	spherical	pure
Upti-prep	Silica	C8U-S	% C : 9	no	60	500	50	spherical	pure
Upti-prep	Silica	C8-S2F	high flow	yes	60	500	140	spherical	pure
Upti-prep	Silica	SAX		no	60	450	60	spherical	pure
Upti-prep	Silica	SCX	0,7 meq/g	no	60	450	60	spherical	pure

# Separation techniques (Proteins)

## Sample Preparation

### Upti-clean S-Series

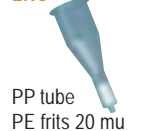
Standard



Straight PP tube  
PE Frits 20 mu

C18-S	C18U	C8	C8U	Weight mg	Volume ml	Qty/pk
C18-S-50/1	C18U-S-50/1	C8-S-50/1	C8U-S-50/1	50	1	50
C18-S-100/1	C18U-S-100/1	C8-S-100/1	C8U-S-100/1	100	1	100
C18-S-100/3	C18U-S-100/3	C8-S-100/3	C8U-S-100/3	100	3	50
C18-S-200/3	C18U-S-200/3	C8-S-200/3	C8U-S-200/3	200	3	50
C18-S-500/3	C18U-S-500/3	C8-S-500/3	C8U-S-500/3	500	3	50
C18-S-500/6	C18U-S-500/6	C8-S-500/6	C8U-S-500/6	500	6	30
C18-S-1G/6	C18U-S-1G/6	C8-S-1G/6	C8U-S-1G/6	1000	6	30
C18-S-2G/6	C18U-S-2G/6	C8-S-2G/6	C8U-S-2G/6	2000	6	20
C18-S-2G/15	C18U-S-2G/15	C8-S-2G/15	C8U-S-2G/15	2000	15	20
C18-S-2G/25	C18U-S-2G/25	C8-S-2G/25	C8U-S-2G/25	2000	25	20

LRC

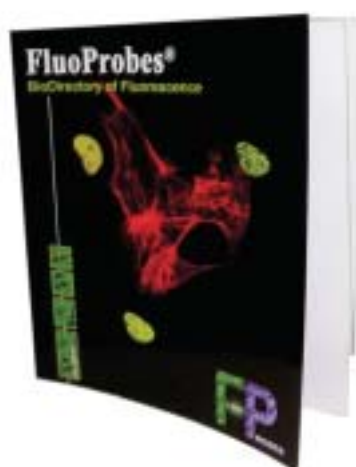


PP tube  
PE frits 20 mu

C18-S-100LRC	C18U-S-100LRC	C8-S-100LRC	C8U-S-100LRC	100	LRC 15	50
C18-S-200LRC	C18U-S-200LRC	C8-S-200LRC	C8U-S-200LRC	200	LRC 15	50
C18-S-500LRC	C18U-S-500LRC	C8-S-500LRC	C8U-S-500LRC	500	LRC 15	50

### Biopolymer

	SPCE	WPCE	Qty ml adsorbant	Vol	Qty/pk
Standard	SPCE-0.5/3	WPCE-0.5/3	0,5	3	50
Straight PP tube	SPCE-1X/3	WPCE-1X/3	1	3	50
PE frits 20 µm	SPCE-2X/6	WPCE-2X/6	2	6	50
	WPAE	SPAЕ			
Standard	WPAE-0.5/3	SPAЕ-0.5/3	0,5	3	50
Straight PP tube	WPAE-1X/3	SPAЕ-1X/3	1	3	50
PE fritS 20 µm	WPAE-2X/6	SPAЕ-2X/6	2	6	50



+ 5500 items / 480 pages

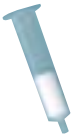
- ◆ Cell Biology Probes (Chap I)
- ◆ Fluorescent Labeling (Chap II)
- ◆ Fluorescent Immunologicals (Chap III)
- ◆ Fluorescent Genetic Tools (Chap IV)
- ◆ Other Fluorescent Tools (Chap V)
- ◆ Custom Services (Chap VI)


gathering the Best of the Fluorescence

FREE Technical Support Center ...  
take the benefit of our Fluorescence knowledge.


# Separation techniques (Proteins)


## Sample Preparation

Standard	SCX	SAX	Weight mg	Volume ml	Qty/pk
 Straight PP tube PE Frits 20 mu	SCX-50/1	SAX-50/1	50	1	50
	SCX-100/1	SAX-100/1	100	1	100
	SCX-100/3	SAX-100/3	100	3	50
	SCX-200/3	SAX-200/3	200	3	50
	SCX-500/3	SAX-500/3	500	3	50
	SCX-500/6	SAX-500/6	500	6	30
	SCX-1G/6	SAX-1G/6	1000	6	30
	SCX-2G/6	SAX-2G/6	2000	6	20
	SCX-2G/15	SAX-2G/15	2000	15	20
	SCX-2G/25	SAX-2G/25	2000	25	20


LRC	SCX	SAX	Weight mg	Volume ml	Qty/pk
 PP tube PE frits 20 mu	SCX-100LRC	SAX-100LRC	100	LRC 15	50
	SCX-200LRC	SAX-200LRC	200	LRC 15	50
	SCX-500LRC	SAX-500LRC	500	LRC 15	50


### Upti-clean S2F-Series

Standard	C18	C18U	C8	Weight mg	Volume ml	Qty/pk
 Straight PP tube PE Frits 20 mu	C18-S2F-100/1	C18U-S2F-100/1	C8-S2F-100/1	100	1	100
	C18-S2F-100/3	C18U-S2F-100/3	C8-S2F-100/3	100	3	50
	C18-S2F-200/3	C18U-S2F-200/3	C8-S2F-200/3	200	3	50
	C18-S2F-500/3	C18U-S2F-500/3	C8-S2F-500/3	500	3	50
	C18-S2F-500/6	C18U-S2F-500/6	C8-S2F-500/6	500	6	30
	C18-S2F-1G/6	C18U-S2F-1G/6	C8-S2F-1G/6	1000	6	30
	C18-S2F-2G/6	C18U-S2F-2G/6	C8-S2F-2G/6	2000	6	20
	C18-S2F-2G/15	C18U-S2F-2G/15	C8-S2F-2G/15	2000	15	20
	C18-S2F-2G/25	C18U-S2F-2G/25	C8-S2F-2G/25	2000	25	20

LRC	C18	C18U	C8	Weight mg	Volume ml	Qty/pk
 PP tube PE frits 20 mu	C18-S2F-100LRC	C18U-S2F-100LRC	C8-S2F-100LRC	100	LRC 15	50
	C18-S2F-200LRC	C18U-S2F-200LRC	C8-S2F-200LRC	200	LRC 15	50
	C18-S2F-500LRC	C18U-S2F-500LRC	C8-S2F-500LRC	500	LRC 15	50

### Atoll polymer based material for SPE

Standard	ATL	ATH	30XC	XC	XWP	Weight mg	Volume ml	Qty/pk
 Straight PP tube PE Frits 20 mu	ATL-30/1	ATH-30/1	30XC-30/1	XC-30/1	XWP-30/1	30	1	50
	ATL-50/1	ATH-50/1	30XC-50/1	XC-50/1	XWP-50/1	50	1	50
	ATL-60/1	ATH-60/1	30XC-60/1	XC-60/1	XWP-60/1	60	1	50
	ATL-75/1	ATH-75/1	30XC-75/1	XC-75/1	XWP-75/1	75	1	50
	ATL-100/1	ATH-100/1	30XC-100/1	XC-100/1	XWP-100/1	100	1	50
	ATL-100/3	ATH-100/3	30XC-100/3	XC-100/3	XWP-100/3	100	3	50
	ATL-150/3	ATH-150/3		XC-150/3	XWP-150/3	150	3	50
	ATL-200/3	ATH-200/3		XC-200/3	XWP-200/3	200	3	50
	ATL-250/3	ATH-250/3		XC-250/3	XWP-250/3	250	3	50
	ATL-500/6	ATH-500/6		XC-500/6	XWP-500/6	500	6	30
	ATL-1G/6	ATH-1G/6		XC-1G/6	XWP-1G/6	1000	6	30

LRC	ATL	ATH	XC	XWP	Weight mg	Volume ml	Qty/pk
 PP tube PE frits 20 mu	ATL-100LRC	ATH-100LRC	XC-100LRC	XWP-100LRC	75	LRC 15	50
	ATL-200LRC	ATH-200LRC	XC-200LRC	XWP-200LRC	150	LRC 15	50
	ATL-500LRC	ATH-500LRC	XC-500LRC	XWP-500LRC	300	LRC 15	50

# Separation techniques (Proteins)

## Sample Preparation

### Micro SPE – UptiTip™

*For preparation of microquantities*

Do not use anymore cartridges or syringes but pipette tips : the sample volumes are dramatically decreased down to 0.1  $\mu$ l.

#### UptiTip™ Coated



New SPE chemistry : pipette tips are directly activated in their inner surface. The surface area in contact with the sample is maximized. Since there is no free materials problems of contamination are avoided.

Features :

- ◆ Faster sample preparation with minimal sample loss
- ◆ No contamination from the support
- ◆ Sample volumes as small as 0.1  $\mu$ l
- ◆ Available in volumes of : 0.1-10  $\mu$ l and 10-200  $\mu$ l

Applications :

- ◆ Desalting
- ◆ MALDI
- ◆ Mass spectroscopy
- ◆ Electrophoresis
- ◆ Protein purification
- ◆ HPCE, HPLC, CEC

#### UptiTip™ Packed

*can also be used as spin column*



Capped tips are filled in with materials. Thanks to the fine slit on the bottom tips (slit width : 1-2  $\mu$ m) the liquid pass through but the chromatographic material (20-30  $\mu$ m) is retained.

One of the main advantage : a filter is not needed so the dead volume is reduced to the minimum. UptiTip™ is excellent for small samples volumes applications.

Revolutionary SPE Micropipette Tips :

Features :

- ◆ Faster sample preparation with minimal sample loss
- ◆ No contamination from the support
- ◆ Sample volumes as small as 0.1  $\mu$ l
- ◆ Available in volumes of : 0.1-10  $\mu$ l and 10-200  $\mu$ l

#### UptiTip™ ChromaLys

*The purification of biological microsamples by using the synergy of 2 effects : Dialysis and Affinity.*

UptiTip ChromaLys™ is a New Concept for : Sample Cleanup, Enzyme Reactions, Affinity Chromatography, Binding Assays & many more Applications.

In a single micropipette tip are combined a dialysis tube and the chromatographic material.

The biological material with a molecular weight smaller than the dialysis tube cut-off go through the membrane and is in contact with the chromatographic material. The unbound material is immediately washed. The bound material is eluted by using the elution buffer. In one step your sample is purified by molecular weight (Dialysis) and by affinity (Chromatographic media).

Last but not least : **your sample is concentrated.**

### UptiTip™-Coated

Chromatographic Media	UptiTip™ Coated (1-10 µl) Cat.# (Pack of 96)	UptiTip™ Coated (10-200 µl) Cat.# (Pack of 96)
C-18	BI5010	BI5020
C-08	BI5030	BI5040
C-04	BI5050	BI5060
HILIC SDS Removal	BI5100	BI5110
PolyCAT A	BI5120	BI5130
SDS-Removal	BI5150	BI1130
<b>Affinity Media</b>		
Silica IMAC	BI5170	BI5180
Ni	BI5190	BI5200
Protein A	BI5210	BI5220
Trypsin	BH3770	BI5230

### UptiTip™-Packed

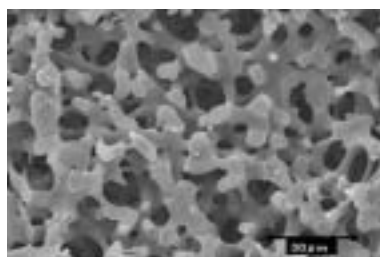
Chromatographic Media	UptiTip™ Packed (1-10 µl) Cat.# (Pack of 96)	UptiTip™ Packed (10-200 µl) Cat.# (Pack of 96)
C-18	BI5270	BI5020
C-08	BI5290	BI5300
C-04	BI5310	BI5320
HILIC SDS removal	BI5390	BI5400
PolyCAT A	BI5410	BI5420
SDS-Removal	BI5440	BI5450
<b>Afinity Media</b>		
Silica IMAC	BI5460	BI5470
Ni	BI5480	BI5500
Protein A	BI5510	BI5520
Protein G	BI5540	BI5560
Lectin ConA	BJ3650	BJ3770
Lectin WGA	BJ3780	BJ3790
Trypsin	BI5570	BI5580

### UptiTip™-ChromaLys

Chromatographic Media	UptiTip™ ChromaLys Media Bed Vol. 1 µl Cat.# (Pack of 20)
C-18	BJ4090
C-08	BJ4140
C-04	BJ4220
HILIC	BJ4310
PolyCAT A	BJ4330
SDS-Removal	BJ4340
<b>Affinity Media</b>	
Silica IMAC	BJ4350
Ni (2+)	BJ4360
Fe (3+)	BJ4370
Lectin ConA	BJ4380
Lectin WGA	BJ4390

# Separation techniques (Proteins)

## Sample Preparation



## Solid Phase Extraction of new generation

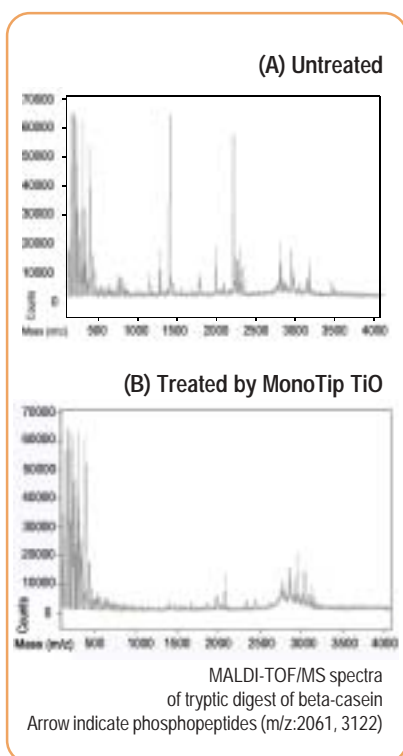
### MonoTip™

Monolithic silica extraction tip (MonoTip™) for sample preparation

MonoTip™ is available to purify and enrich femto mole to micro mole of peptides and proteins before MALDI-MS and LC-MS analysis. Monolithic silica consist of double pore structure :

- ◆ Continuous through-pores
- ◆ Silica skeleton with meso pores

The unique monolithic silica structure contributes to low pressure drop and strong analyte to surface interactions. In MonoTip™, monolithic silica is directly attached to the inner surface of the 10 μl tip and 200 μl tip.



### MonoTip™ C18

- ◆ Faster sample preparation
- ◆ Low sample loss
- ◆ No contamination from monolithic silica
- ◆ Large sample capacity

### MonoTip™ C18

Silica type : High purity sol-gel silica gel  
Specific surface area : 200 m<sup>2</sup>/g  
Through pore size: 10 – 20 μm  
Meso pore size : 20 nm  
Bonded phase : Octadecyl group  
Carbon load : 12%  
Tip volume : 200 μl  
Sample capacity : 100 μg (Angiotensin II)  
Maximum MW : MW 40000

### MonoTip™ mini C18

Silica type : High purity sol-gel silica gel  
Specific surface area : 200 m<sup>2</sup>/g  
Through pore size: 20 – 30 μm  
Meso pore size : 15 nm  
Bonded phase : Octadecyl group  
Carbon load : 12%  
Tip volume : 10 μl  
Sample capacity : 5 μg (Angiotensin II)  
Maximum MW : MW 5000

### MonoTip™ Titania

Phosphopeptides purification and enrichment

In the field of HPLC, it is reported that the columns packed with Titanium Dioxide (TiO<sub>2</sub>) particles was applied for the analysis of phospho-compounds [1-4]. MonoTip™ TiO is enable to capture selectively phosphopeptides from a comparatively large background of unmodified peptides. MonoTip™ TiO is a highly efficient and versatile tool to purify phosphorylated peptides from proteolytic digests prior mass analysis.

Feature :

- ◆ Faster sample preparation
- ◆ Low sample loss
- ◆ High selectivity

Literature :

- [1]-Pinkse MW, Uitto PM, Hilhorst MJ, Ooms B, Heck AJ, Anal. Chem. 2004, 76, 3935-43
- [2]-Kuroda I, Shintani Y, Motokawa M, Abe S, Furuno M, Anal Sci. 2004, 20, 1313-93
- [3]-Kimura Y, Shibasaki S, Morisato K, Minakuchi H, Nakanishi K, Matsuo M, Amachi T, Ueda M, Ueda K, Analytical Biochem. 2004, 326, 262-266
- [4]-Sekiguchi Y, Mitsuhashi N, Mimura T, Plant Biotechnology 2004, 21, 143-150
- [5]- Miyazaki S, Mirosato K, Suzuki K, Nakanishi K, Ueda M, 53rd ASMS, 2005

### MonoTip™ TiO

Monolith : Titania coated high purity sol-gel silica gel  
 Specific surface area : 200 m<sup>2</sup>/g  
 Through pore size: 10 – 20 µm  
 Meso pore size : 20 nm  
 Tip volume : 200 µl  
 Sample capacity : 15 µg (Tyrosine phosphopeptide)

Insérer Chromato PP Monotip TiO

### MonoTip™ Trypsin

Protein digestion

MonoTip™ Trypsin (TPCK treated Bovine Pancreas immobilized on monolith silica) catalyzed quick digestion of reduced and alkylated proteins with merely few times operation at room temperature.

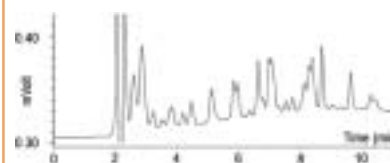
- ◆ Faster sample preparation enzyme action about aspirate/expel 10x at room temperature
- ◆ Low sample loss
- ◆ No contamination from self digestion of trypsin and monolithic silica
- ◆ Large sample capacity (100 µg protein)

### MonoTip™ Trypsin

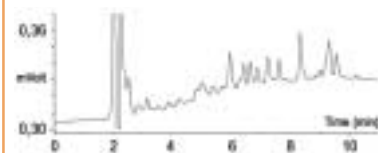
Silica type : High purity sol-gel silica gel  
 Specific surface area : 100 m<sup>2</sup>/g  
 Through pore size: 10 – 20 µm  
 Meso pore size : 30 nm  
 Tip volume : 200 µl  
 Sample capacity : 100 µg (Denaturated BSA)  
 Enzyme Buffer : 50 mM Ammonium bicarbonate pH8.0

### Large sample capacity

1mg/ml  
 Denaturated BSA 100 µg (after Digestion)

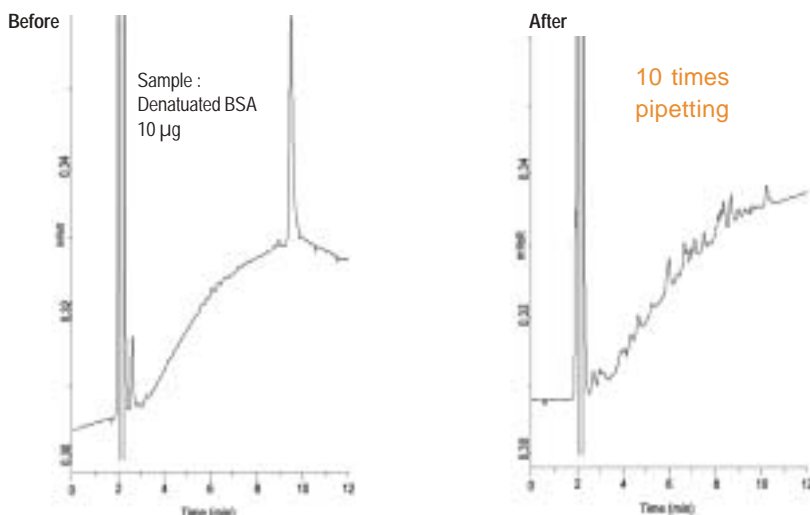


1mg/ml  
 Denaturated Transferrin 100 µg (after Digestion)



Condition  
 Column : Inertsil WP300 C8 (5 m,150 x 4.6mm I.D.)  
 Eluent :  
 A) 0.1%TFA in H<sub>2</sub>O  
 B) 0.1%TFA in CH<sub>3</sub>CN  
 A/B=90/10-10min-40/60  
 Flow rate : 1mL/min  
 Col.Temp.: 35  
 Detection : UV 210nm

### Digestion by few times pipetting



Condition  
 Column : Inertsil WP300 C8 (5 m,150 x 4.6mm I.D.)  
 Eluent : A) 0.1%TFA in H<sub>2</sub>O  
 B) 0.1%TFA in CH<sub>3</sub>CN  
 Gradient : A/B=90/10-10min-40/60

Flow rate : 1mL/min  
 Col.Temp.: 35  
 Detection : UV 210nm

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## Sample Preparation

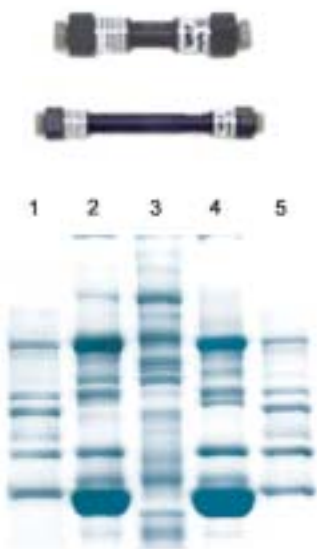
### Affinity Extraction

#### Multiple Affinity Removal System (MARS)

Tools to remove unwanted multiple high-abundant proteins with very high specificity. These tools are based on the affinity of an antibody with its antigen. The highly specific antibodies are covalently bonded on the resin and are available to trap the antigens. The covalently bonded chemistry avoids any leakage of the antibodies allowing numerous uses of the support without any decreasing efficiency. The systems are available in columns or in cartridge format.

#### Human Serum Column

For the first time, you can identify and characterize high-value, low-abundant proteins faster and more efficiently without interferences. The Agilent Multiple Affinity Removal System Human Serum Column allows you to remove unwanted multiple high-abundant proteins with very high specificity from human serum, plasma, CSF samples, and more — with just one device.



- ◆ Removes six targeted high-abundant proteins: albumin, IgG, antitrypsin, IgA, transferrin, and haptoglobin (85–90 percent of total serum protein mass).
- ◆ Improves the results you get with protein separation methods such as 1DGE, 2DGE, and MD-HPLC.
- ◆ Goes beyond the limitations of other protein-depletion devices.
- ◆ Expands the dynamic range of current LC/MS and electrophoretic analytical methods.
- ◆ Can be reused for at least 200 injections.
- ◆ Minimizes sample loss by using just one column for removing multiple proteins.

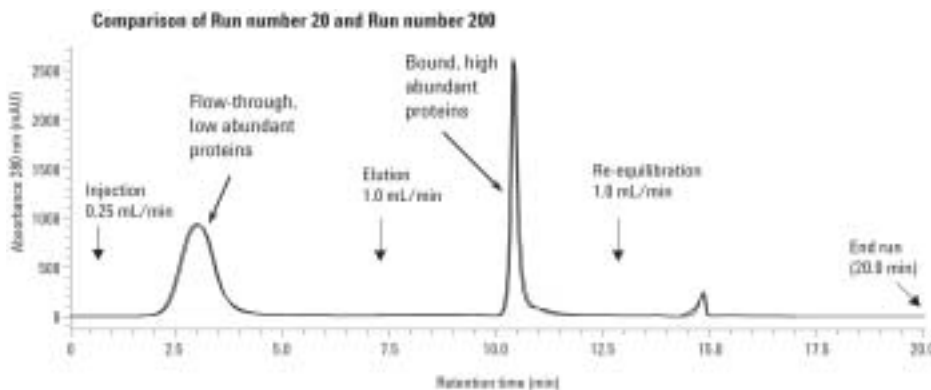
The Multiple Affinity Removal System are very specific, this minimizes the chance of removing proteins of interest along with the targeted proteins, which occurs often with competitive devices.

Also available in cartridge format p.B.190.

New

#### High-Capacity Human Serum Column

With these new columns, you get more capacity for your money, and higher capacity is needed to facilitate «digging deeper» into the proteome. Agilent now offers high-capacity versions of the Multiple Affinity Removal System for human serum proteins — giving you a better value for your investment and the tools you need to dig deeper into the proteome. Improved technology enables at least twice the capacity for removing human proteins with high-capacity columns (compared to standard columns), while offering the same high selectivity and immunodepletion efficiency you expect with Agilent immunodepletion devices. These columns are reusable for greater than 200 runs.



Runs #1 and #200 for human serum on a 4.6 x 50 mm High Capacity Multiple Affinity Removal Column. High reproducibility and longevity as indicated above makes immunodepletion with these affinity columns ideal for comparing low-abundance protein fractions from many serum samples in biomarker studies.

### Mouse Serum Column

The Multiple Affinity Removal System Mouse Serum Column is the first multiple high-abundance protein-removal LC column specific for mouse and rat serum. It simultaneously targets and removes 98-99% of three high-abundant proteins (Albumin, Transferrin and IgG) from mouse and rat serum samples, with a higher specificity and efficiency than other protein-removal devices. This column complements our line of human serum protein-removal.

- ◆ More specific to targeted proteins than dye-based affinity media
- ◆ Expands range of low-abundant proteins by gel electrophoresis and LC/MS
- ◆ Enables potential protein biomarker identification for disease or drug targets
- ◆ Exhibits lower non-specific protein binding

### MARS Columns descriptions :

<b>Column dimensions</b>	4.6 mm x 50 mm (0.83 mL bed volume) 4.6 mm x 100 mm (1.66 mL bed volume)
<b>Column body material</b>	PEEK (polyetheretherketone)
<b>End-Fitting material</b>	PEEK with 2 µm frits
<b>Column capacity**</b>	Standard sample load
	50 mm column Hu-6 : 15-20 µL human serum
	50 mm column Mu-3 : 37-50 µL mouse serum
	100 mm column Hu-6 : 30-40 µL human serum
	100 mm column Mu-3 : 75-100 µL mouse serum
<b>New</b>	High-capacity sample load (human only)
	50 mm column Hu-6HC : 30-40 µL human serum
	100 mm column Hu-6HC : 80-100 µL human serum
<b>Max. pressure</b>	120 bar
<b>Operating temperature</b>	18 - 25°C
<b>Column packing material</b>	Affinity resin
<b>Immobilized ligands</b>	Affinity ligands to human (albumin, IgG, antitrypsin, IgA, transferrin and haptoglobin) or mouse high abundant proteins (albumin, IgG, and transferrin)
<b>Flow rate range</b>	0.25 - 1.0 mL/min
<b>Shipping solution</b>	Buffer A with 0.02% sodium azide
<b>Shipping temperature</b>	2 - 8°C - (35 -46°F)
<b>Storage temperature</b>	2 - 8°C - (35 -46°F)

### Capacity :

	Protein	Column ID (cm)	Column length (cm)	Column volume (mL)	# Runs per column life
Human	All 6	0,46	5	0,83 15-20 µL serum	200
	All 6	0,46	10	1,66 30-40 µL serum	200

### MARS ordering info :

Column	Cat.#
Mult Aff Rem Column 4.6 x 50 mm Hu-6	5185-5984
Mult Aff Rem Column 4.6 x 100 mm Hu-6	5185-5985
Mult Aff Rem Column 4.6 x 50 mm Ms-3	5188-5217
Mult Aff Rem Column 4.6 x 100 mm Ms-3	5188-5218
Mult Aff Rem Column 4.6 x 50 mm Hu-6 High-Capacity	5188-5332
Mult Aff Rem Column 4.6 x 100 mm Hu-6 High-Capacity	5188-5333

\*Rat serum capacity is ~50-60% of mouse serum capacity.



\*\*For exact column capacity, consult your column certificate of analysis. Human and mouse sera protein concentrations can vary; injected sample sizes should be adjusted accordingly. Mouse serum capacities were determined using pooled Swiss Webster mouse serum.

Also available in cartridge format page B190

# Separation techniques (Proteins)

## Sample Preparation



\*\* For exact spin cartridge capacity, consult your spin cartridge certificate of analysis. Human and mouse sera protein concentrations can vary; sample sizes should be adjusted accordingly. Mouse serum capacities were determined using pooled Swiss Webster mouse serum.

### Cartridges

These spin cartridges offer the same protein removal technology as that used in the Multiple Affinity Removal LC columns, however serum and plasma samples can now be processed by using standard benchtop microcentrifuge equipment and a disposable syringe rather than an HPLC instrument.

- ◆ Use of a benchtop microcentrifuge
- ◆ Multiple samples are processed at one time
- ◆ Cartridges reusable at least 200 times

With these tools 80-90% of total protein mass (albumins, IgG, antitrypsin, IgA, transferrin and haptoglobin from human serum) (Albumin, Transferrin and IgG from mouse and rat serum) are depleted. Last thing, a complete depletion and regeneration of the columns are performed in approximately 10 minutes.

The Spin cartridges for human are available in 2 formats, the normal and the high capacities.

<b>Cartridge Volume</b>	0.45 mL resin in 1 mL tube
<b>Cartridge body material</b>	Polypropylene
<b>Frit materials</b>	Polyethylene with 10 µm pore size
<b>Cartridge capacity**</b>	Standard sample load
	0.45 mL cartridge Hu-6 : 7-10 µL human serum
	0.45 mL cartridge Ms-6 : 25-30 µL mouse serum
<b>New</b>	High-capacity sample load (human only)
	0.45 mL cartridge Hu-6HC : 14-16 µL human serum
<b>Recommended centrifugal force</b>	100 x g
<b>Operating temperature</b>	18 - 25°C
<b>Cartridge packing material</b>	Affinity resin (0.45 mL)
<b>Immobilized ligands</b>	Affinity ligands to human (albumin, IgG, antitrypsin, IgA, transferrin and haptoglobin) or mouse high abundant proteins (albumin, IgG, and transferrin)
<b>Shipping solution</b>	Buffer A with 0.02% sodium azide
<b>Shipping temperature</b>	2 - 8°C - (35 -46°F)
<b>Storage temperature</b>	2 - 8°C - (35 -46°F)

Cartridges	Cat.#
Mult Aff Rem Spin Cart. 0.45 mL Hu-6	5188-5230
Mult Aff Rem Spin Cart. 0.45 mL Hu-6 High Capacity	5188-5341
Mult Aff Rem Spin Cart. 0.45 mL Ms-3	5188-5289