Interchim Innovations

Interbiotech - BioScience Innovations

Norgen Proteomics tools

Cell Lysis Reagent & Inclusion Body Protein Solubilization Reagent	
Inclusion Body Protein Purification kit	for research use
Protein clean-up, Concentration and Endotoxin Removal	for research and diagnostic use
Urine Protein isolation and concentration kits	
Depletion of Abundant Serum Proteins	for research and diagnostic use
On-Column Proteolytic Digestion	for research use

Cell Lysis & Inclusion Body Solubilization Rgts

Cell Lysis Reagent

For the efficient lysis of bacterial cells and the extraction of inclusion body proteins

Cell Lysis Reagent **18800, 100mL**

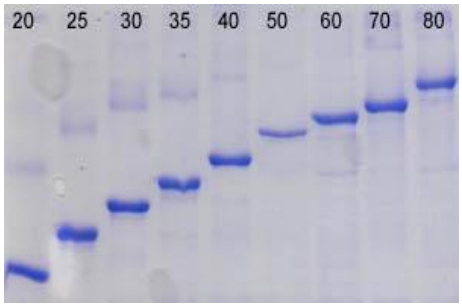

Figure 3. Efficient Isolation of Inclusion Body Proteins

- Proprietary solution of detergents, protease-inhibitors and buffer
- Efficient lysis of bacterial cells for extraction of inclusion body proteins

The Cell Lysis Reagent is designed for the gentle and efficient lysis of bacterial cells in order to assist in the extraction of inclusion body proteins. Cell lysis is accomplished through non-ionic detergent chemical disruption in conjunction with mechanical disruption. The use of a needle and syringe during the procedure helps to reduce viscosity and facilitate purification, producing proteins that are often greater than 95% pure.

Cell Lysis Reagent does not solubilize inclusion bodies.

• Documentation

Technical Sheet 57200 (FT-1Q0780)

57200 - Protocol (48 reactions)(FT-1Q0781)

Application note :

Comparing Different DNA and RNA Quantification Methods for Biological Samples with Low Nucleic Acid Abundance

Customer-supplied Reagents and Equipment:

Centrifuge
Micropipettors
Needles (20 guage for small scale protocol and 18 guage for large scale protocol)
Syringes (1 mL for small scale protocol, 10 mL for large scale protocol)
Sterile deionized or dezionized water

Inclusion Body Solubilization Reagent

For the efficient solubilization of inclusion body proteins

Inclusion Body Solubilization Reagent 18700, 25 mL 18701, 100 mL

- Rapid and convenient solubilization of inclusion body aggregates
- Allows for downstream processing of dissolved inclusion body proteins

The Inclusion Body Solubilization Reagent dissolves inclusion body aggregates resulting from the expression of recombinant proteins in bacteria. This reagent has demonstrated exceptional ablility to solubilize inclusion bodies. Once dissolved, proteins can be analyzed by SDS-PAGE, quantified or further purified for refolding.

Inclusion bodies can be released from bacterial cells usingCell Lysis Reagent (available separately as #18800). Inclusion Body Protein Isolation Kits can also be used as complete kits #1700&17 for cell disruption, inclusion body solubilisation and purification using spin column chromatography

• Documentation

Technical Sheet (FT-1F2400)

18700, 18701 - Safety Data Sheet

Inclusion Body Protein Purification Kits

These Kits facilitates the screening of E. coli clones that express recombinant proteins in inclusion bodies. Specially formulated reagents achieve rapid and high-quality purification of inclusion body proteins using three processes:

1-Lysis of bacterial cells to release inclusion bodies in solid form
2-Solubilization of purified inclusion bodies
3-Purification of the recombinant protein using spin column chromatography

ProteoSpin™ Inclusion Body Protein Isolation Micro Kit 10300, 20 preps (Lv)
ProteoSpin™ Inclusion Body Protein Isolation Maxi Kit 17700, 4 preps (Lv)
Cell Lysis Reagent 18800, 100 m
Inclusion Body Solubilization Reagent 18700, 25 mL 18701, 100 mL (Lv)
Kits for Research Use

The ProteoSpin™ Inclusion Body Isolation Kits provide essential reagents for cell disruption, inclusion body solublization and purification using spin column chromatography. The kit includes solutions and protocols for use with either acidic or basic proteins.

ProteoSpin™ Inclusion Body Protein Isolation Micro Kit (Cat. 10300)

For the rapid isolation of inclusion body proteins - cells to gels in 60 minutes

- All-in-one solution for inclusion body protein isolation and purification
  Complete kit with Cell Lysis Reagent, Inclusion Body Solubilization Reagent, buffers and spin columns to purify proteins
- Fast and convenient spin column protocol
- Complete kit with Cell Lysis Reagent, Inclusion Body Solubilization Reagent, buffers and spin columns to purify proteins
- Isolate up to 12 mg of protein per spin column (Maxi Kit)

ProteoSpin™ Inclusion Body Protein Isolation Maxi Kit 17700, 4 preps (Lv)
ProteoSpin™ Inclusion Body Protein Isolation Micro Kit 17700, 20 preps (Lv)

Kits content : 1.Wash Solution C ; 2.Wash Solution N3.Binding Buffer A; 4.Binding Buffer N; 5.Elution Buffer C; 6.Protein Neutralizer; 7.Cell Lysis Reagent; 8.IB Solubilization Reagent; 9.Syringes, 1cc, slip tip; 10.Needles (Bev, 20G x 1 inch); 11.Micro or Macro Spin Columns; with Collection/Elution Tubes(1.7 mL or 50mL); 14.Product Insert

• Kit Specifications	Micro Kit	Maxi Kit
Maximum Culture Volume	1.5 mL	100mL
Yield from 1.5 mL	Up to 50 µg	Up tot 12mg
Minimum Elution Volume	30 µL	4mL
Time to Process 12 Samples	60 minutes	1 Hour

• Benefits of the ProteoSpin™ Inclusion Body Isolation Micro Kit

Three kits in one for acidic and basic proteins	The kit provides essential reagents for cell disruption, inclusion body solublization and purification using spin column chromatography. The kit includes solutions and protocols for use with either acidic or basic proteins.
Gentle disruption procedure provides high purity final product.	Cell lysis is accomplished through non-ionic detergent chemical disruption. The use of a needle and syringe reduces viscosity and facilitates purification, producing proteins that are often >95% pure. Proteins are then ready for SDS-PAGE, 2D gels, mass spectrometry analysis or other procedures.
Convenient process volume	Analyze 1.5 mL of microbial cell culture yielding 2-50 µg of protein.Fast and easy processingProcess up to 12 samples in only 60 minutes
Long kit shelf life	The kit uses an enzyme-free process with stable solutions for longer shelf life.
Suitable for downstream applications	Final elution performed with volatile or non-volatile buffers suitable for: Mass spectrometry SDS-PAGE Refolding experiments

• Kits results

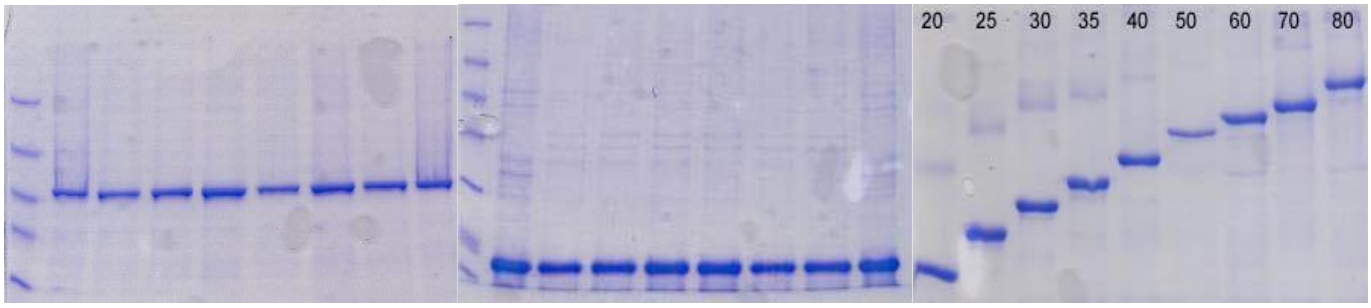

Figure 1. Isolation of Acidic Recombinant Proteins

Isolation of a recombinant 50 kDa chimeric protein (acidic) using the ProteoSpin™ Inclusion Body Iso-lation Micro Kit.

Figure 2. Isolation of Basic Recombinant Proteins

Isolation of recombinant RNaseA (basic protein) using the ProteoSpin™ Inclusion Body Isolation Micro Kit.

Figure 3. Efficient Isolation of Inclusion Body Proteins

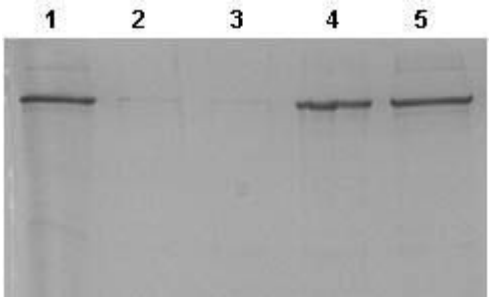

Figure 1. No Loss of Proteins when Isolating a Basic Protein

ProteoSpin™ Inclusion Body Protein Isolation Micro Kit

This kit provides everything required to isolate and purify inclusion body proteins from induced bacterial cultures. First a proprietary Cell Lysis Reagent is used to selectively lyse the cells and release inclusion bodies in their solid form. Next, inclusion bodies are dissolved and their contents released using the provided IB Solubilization Reagent. Inclusion body proteins are then further purified using spin columns for rapid and convenient buffer exchange and desalting. This kit provides a convenient way to screen recombinants prior to scaling up.

Maxi Kit : The procedure is efficient and streamlined and can process up to 4 samples in approximately 2 hours. Each spin column is able to recover up to 12 mg of acidic or basic proteins from 100 mL of induced bacterial culture. Purified recombinant proteins are then ready for SDS-PAGE, 2D gels, Western blots, Mass Spectrometry analysis, and other applications.
Micro Kit : The process is efficient and streamlined and can process up to 12 samples in only 60 minutes. Each spin column is able to recover up to 50 µg of acidic or basic proteins. Purified recombinant proteins are then ready for SDS-PAGE, 2D gels, Western blots, Mass Spectrometry analysis, and other applications.

About Inclusion Bodies

Bacteria are widely used for the expression of different proteins. However, 70-80% of the proteins expressed in bacteria by recombinant techniques are typically contained in insoluble inclusion bodies (i.e., protein aggregates). The protein of interest found in these sub-cellular structures is often inactive, due to incorrect folding. The production rate of recombinant proteins stored in inclusion bodies is invariably higher than those synthesized as soluble proteins. The reason behind this is thought to be the resistance of insoluble proteins to proteolysis by cellular enzymes. In addition, separation of insoluble recombinant proteins in inclusion bodies is considerably easier than that of soluble proteins. These factors have been the major influences favoring scale-up of high-value proteins using bacterial fermentation for example. Procedures for the purification of the expressed proteins from inclusion bodies are often labour-intensive, time consuming and not cost effective.

This kit provides the essential reagents for cell disruption, inclusion body solubilization and purification using spin column chromatography - all optimized to work together thereby simplifying the process and saving a tremendous amount of time and cost.

• Documentation

Protocols:

Technical Sheet 10300-17700 (1J8620) Product Information Sheet of ProteoSpin Inclusion Body Protein Isolation Micro Kit
Product Insert 10300-17700 (1J8621) -10300 - Protocol (20 prep)
Technical Sheet 17700 (A2Z1U0) Product Information Sheet of ProteoSpin Inclusion Body Protein Isolation maxi Kit
Product Insert 17700 (A2Z1U1) -17700 - Protocol (20 prep)

Application Notes:

Screening For Bacterial Clones Expressing Inclusion Body Proteins
A Time Course Induction Study of Recombinant Protein Expression In E Coli

Safety Data Sheets: 10300 - Safety Data Sheet 17700 - Safety Data Sheet

• Storage

The Cell Lysis Reagent and the IB Solubilization Reagent should be stored at 4°C upon receipt of the kit. All other unopened solutions should be stored at room temperature. Once opened, the solutions should be stored at 4°C when not in use, except for the Basic and Acidic Binding Buffers, which should be stored at room temperature.

ProteoSpin™ Total Protein Concentration, Detergent Clean-Up and Endotoxin Removal Mini Kit (Cat. 22800)

For rapid and efficient endotoxin removal from proteins and peptides

- Columns bind proteins of interest while endotoxins flow through
- Proteins are desalted
- Reduce endotoxin levels to less than 0.01 EU/µg of protein
- Greater than 95% protein recovery
- Concentrate protein samples and remove detergents at the same time
- Effectively remove a wide range of detergents including SDS, Triton® X-100, CHAPS, NP-40, and Tween 20

ProteoSpin™ Total Protein Concentration, Detergent Clean-Up and Endotoxin Removal Mini Kit 22800, 25 preps

• Kits results

Figure 1. **Endotoxin-Free Proteins**

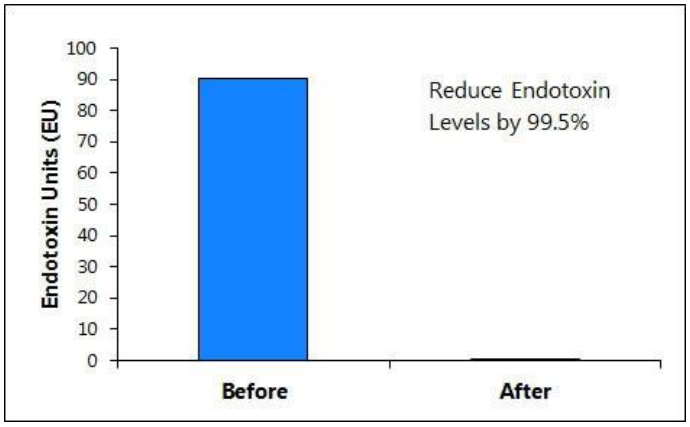

Figure 2. **High Protein Recovery**

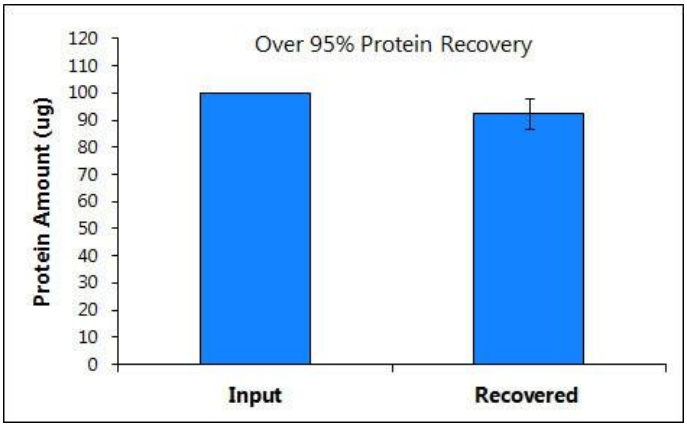

*Figure 3. **Efficient Removal of SDS** from Lysate

Input Sample	% SDS	% SDS removal
Bacterial Lysate	1.0%	100%
Mammalian Lysate	0.1%	99.998%

*Figure 4. Concentration of BSA

Input volume	Output volume	Concentration factor
1mL	50µL	20X
3mL	50µL	60x
5mL	50µL	100x

Figure 8. **High Protein Recovery in presence of SDS**

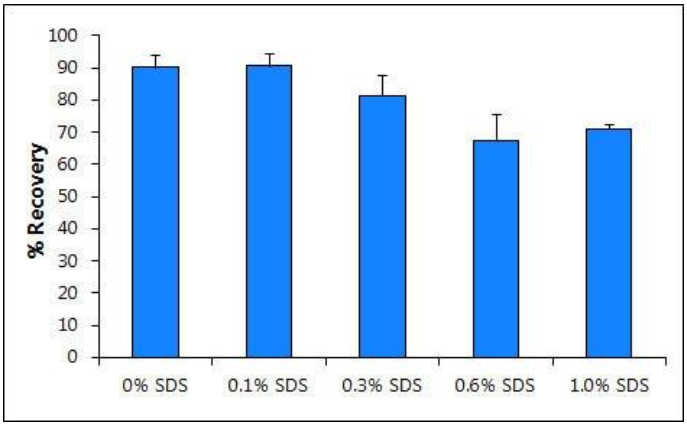

*Figure 5. **Concentration of BSA**

Input Concentration	Output Concentration	**Concentration Factor**
50µg/2µl (0.025µgml)	46µg/50µL (0.96µg/ml)	**38x**

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*Figure 6. **Protein Recovery and Salt Reduction**

Salt (300ppm)	% BSA recovery	**% Salt Reduction**	Salt (300ppm)	% BSA recovery	**% Salt Reduction**
$MgCl_2$	68.4	**99.7%**	KCl	76.2	**99.0%**
NaCl	70.9	**96.1%**	$CaCl_2$	69.6	**96.4%**

*Figure 7. **Efficient Removal of SDS**

Sample	A600nm	%SDS remaining	**% SDS removal**	**mg SDS removed**
Control (water)	0	0	0	0
0.1% SDS	0.142		()	
ProteoSpin Sample1	0.024	0.017%	**99.98%**	**2.457**
ProteoSpin Sample2	0.030	0.02%	**99.98%**	**2.458**

• **Kit Specifications**	**Mini Kit**	**Maxi kit**
Maximum Protein Input	200 µg	4mg (min.0.25mg)
Maximum Column Volume Input	600 µL	18ml
Molecular Weight of Recovered Proteins	No MWCO	No MWCO
Final Endotoxin Levels	≤ 0.01 EU/µg protein	≤ 0.01 EU/µg protein
Protein Recovery	90-95%	90-95%
% Detergent Removal	90-95%	90-95%
Detergents that can be Removed	Including Triton® X-100, CHAPS, NP-40 and Tween 20	
Minimum Elution Volume	50 µL	2.4mL
Time to Complete 10 Purifications	20 minutes	30-40 minutes

• **Documentation**

Protocols:

Technical Sheet 10300-17700 (1J8620) Product Information Sheet of ProteoSpin Inclusion Body Protein Isolation Micro Kit
Product Insert 10300-17700 (1J8621) -10300 - Protocol (20 prep)
Technical Sheet 17700 (A2Z1U0) Product Information Sheet of ProteoSpin Inclusion Body Protein Isolation maxi Kit
Product Insert 17700 (A2Z1U1) -17700 - Protocol (20 prep)

Application Notes:

Screening For Bacterial Clones Expressing Inclusion Body Proteins
A Time Course Induction Study of Recombinant Protein Expression In E Coli

Safety Data Sheets:

See also Urine Protein Concentration Kits

ProteoSpin™ Urine Protein Concentration Micro Kit	**17400, 25 preps**
ProteoSpin™ Urine Protein Concentration Midi Kit	**52300, 10 preps**
Detergent-Free Total Protein Isolation Kit	**21600, 4 preps**

ProteoSpin™ Abundant Serum Protein Depletion Kit (Cat. 17300)

For the rapid depletion of abundant proteins from serum samples

- Rapid and efficient removal of abundant proteins from serum and plasma samples
- Process 10 samples in 30 minutes
- Allows for visualization of low abundance proteins
- Convenient and affordable spin column protocol
- Generic protocol allows for depletion from human and other animal samples

ProteoSpin™ Abundant Serum Protein Depletion Kit 17300, 25 preps

• Kits results

Figure 1. **Depletion of Abundant Proteins**

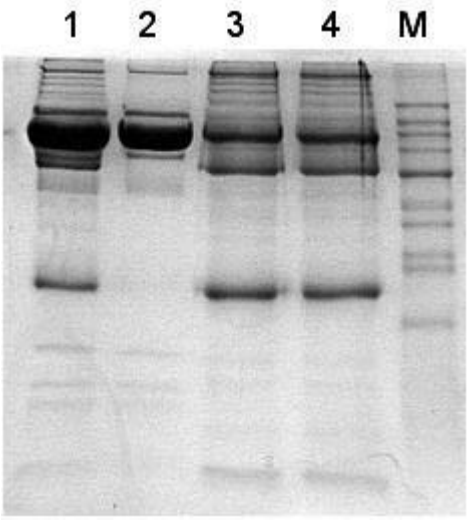
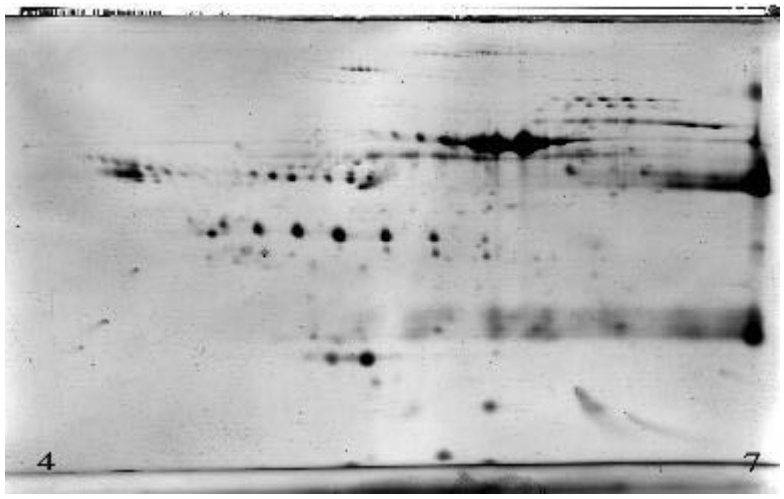


Figure 2. Depletion of Abundant Proteins



This kit provides a fast and simple procedure for the effective depletion of major serum proteins including albumin, α-antitrypsin, transferrin and haptoglobin from serum and plasma samples. Such abundant proteins usually obscure less abundant proteins in gel electrophresis making them difficult to visualize and recover. The kit reduces sample complexity to allow for convenient investigation of less abundant proteins. In other applications such as mass spectroscopy, fractionating abundant proteins improves the resolution of less abundant proteins. The kit is 'generic' in that it is not antibody-based and can thus be used to deplete abundant proteins from any starting material - human or various other animals - making it more versatile, flexible and affordable than other kits. Resulting proteins are ready for a range of applications including 2D gel electrophoresis, SDS-PAGE, Mass Spectrometry, Protein microarrays, DIGE (Difference Gel Electrophoresis) and more.

• Kit Specifications

Maximum Column Capacity	150 µg
Minimum Column Capacity	50 µg
Minimum Elution Volume	30 µL
Time to Process 10 Samples	30 minutes

• Documentation

Protocols: Technical Sheet 17300 (1F2410) Product Information Sheet of ProteoSpin™ Abundant Serum Protein Depletion Kit
Product Insert 17300 (1F2411) - Protocol (20 prep)

Safety: 17300 - Safety Data Sheet

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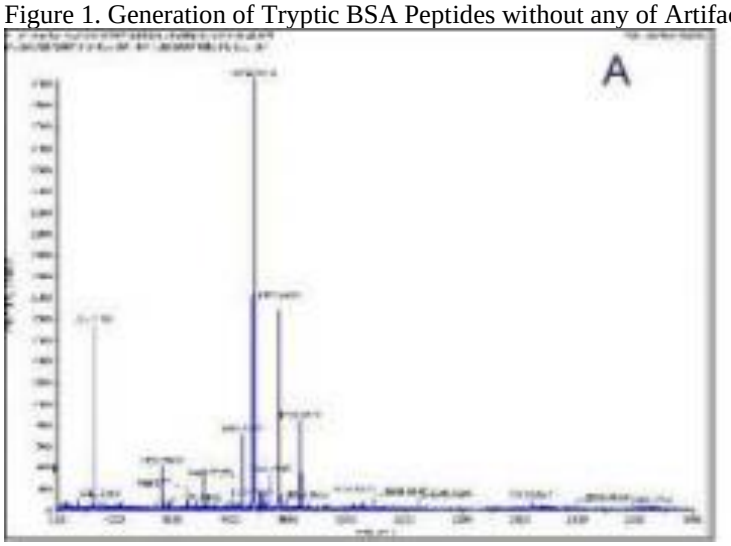
ProteoSpin™ Digestion Kit

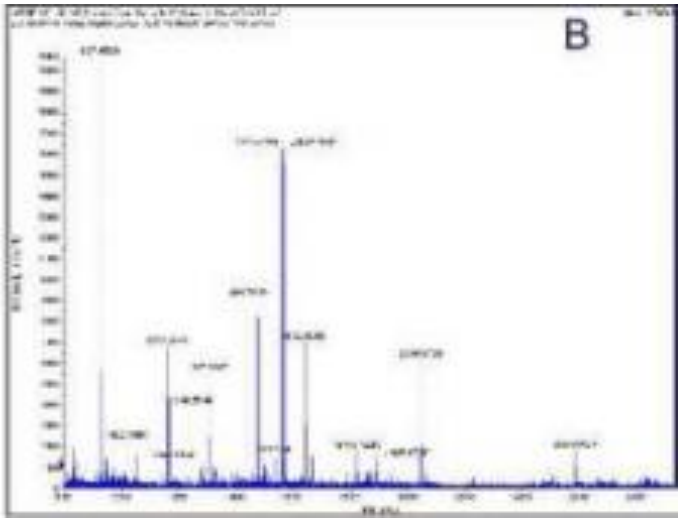
- Rapid and simple procedure to generate digested peptides
- **Simultaneous digestion, purification and concentration at once**
- Peptide generation is **complete**, with **no generation of additional artifacts** being detected in mass spectrometry
- Peptides are ready for applications such as mass spectrometry and SDS-PAGE

ProteoSpin™ On-Column Proteolytic Digestion Kit **17500** **25 preps**

• Kits results

Figure 1. Generation of Tryptic BSA Peptides without any of Artifacts.





ProteoSpin™ On-Column Proteolytic Digestion Kit is highly efficient in the enzymatic digestion of simple and complex protein samples using trypsin and the subsequent purification of the resulting peptides using a convenient spin column format. Trypsin is added to the protein sample and bound to the column. Salts are washed away and the trypsin is then activated to digest proteins. Peptides are then eluted in a small volume and ready for downstream analysis. The peptides generated are complete, with no additional artifacts being detected in mass spectrometry. Fifteen micrograms of protein can be processed, digested and purified with each spin column with about 20 minutes of hands-on time (plus trypsin incubation). The simultaneous protein digestion and volumetric concentration of the purified peptides makes the kit a convenient method for preparing peptides to be analyzed by many downstream applications such as mass spectrometry and more.

• Documentation

Protocols: Technical Sheet 17500 (FT-A2Z1I0) Product Information Sheet of ProteoSpin™ Abundant Serum Protein Depletion Kit
Product Insert 17500 (FT-A2Z1I1)

Safety: 17500 - Safety Data Sheet - Protocol (25 prep)

Citations:

Title	Clinical Proteomics: Liquid Chromatography–Mass Spectrometry (LC–MS) Purification Systems
Journal	Protein Chromatography. 2016.
Authors	Henry M, Meleady P.
Title	Neonatal NET-inhibitory factor and related peptides inhibit neutrophil extracellular trap formation
Journal	The Journal of Clinical Investigation. 2016.
Authors	Yost CC, et all
Title	Doublecortin-Like Kinase 1 Is Elevated Serologically in Pancreatic Ductal Adenocarcinoma and Widely Expressed on Circulating Tumor Cells
Journal	PLoS ONE. 2015.
Authors	Dongfeng Qu, et all
Title	Identification of plasma Complement C3 as a potential biomarker for neuroblastoma using a quantitative proteomic approach.
Journal	Journal of Proteomics. 2013.

Authors	Kim PY, et all .
Title	Quantitation of irbesartan and major proteins in human plasma by mass spectrometry with time-of-flight analyzer.
Journal	Journal of Pharmaceutical and Biomedical Analysis. 2011.
Authors	Lu CY, Feng CH.
Title	Proteome analysis of rat serum proteins adsorbed onto synthetic octacalcium phosphate crystals.
Journal	Analytical Biochemistry. 2010.
Authors	Kanekoa H, Kamiieb J, Kawakamib H, Anadac T, Hondac Y, Shiraishid N, Kamakurae S, Terasakif T, Shimauchia H, Suzukic O.
Title	Modification of major plasma proteins by acrylamide and glycidamide: screening by nano liquid chromatography with tandem mass spectrometry.
Journal	Analytica Chimica Acta. 2010.
Authors	Chia-Hsien Fenga and Chi-Yu Lu.
Title	Analysis of angiotensin II receptor antagonist and protein markers at microliter level plasma by LC?MS/MS
Journal	Journal of Pharmaceutical and Biomedical Analysis.49 123?128. 2009.
Authors	Lu CY, Chang YM, Tseng WL, Feng CH, Lu CY.

Application notes Ask

•Rapid Concentration of Dilute Protein Solutions Using the ProteoSpin™ CBED Kit

•Rapid Desalting of Protein Solutions Using the ProteoSpin™ CBED Kit

•Efficient Removal of Both Free and Tightly-Bound SDS from Protein Solutions using the ProteoSpin Detergent Clean-up Kit

•Efficient Removal of Triton X-100 Prior to MALDI-TOF and Digestion Using the ProteoSpin Detergent Clean-up Kit

•Screening for Bacterial Clones Expressing Inclusion Body Proteins

•A Time Course Induction Study of Recombinant Protein Expression in E.coli

•Sequential Isolation and Purification of RNA, DNA, and Protein from a Single Sample of Animal Cells using Norgen's RNA/DNA/Protein Purification Kit

•Sequential Purification of RNA, DNA, and Protein from a Single Sample using Norgen's RNA/DNA/Protein Purification Kit and Comparison to a Market Competitor

•The Range of Protein Yield with Norgen's Urine Protein Concentration, Preservation, and Isolation Kit

Related products/documents

BB101a Dialysis selection guide
BB117s Vivacon & VivaSpin centrifugal concentration devices, by Ultrafiltration
Products HighLights Overview

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