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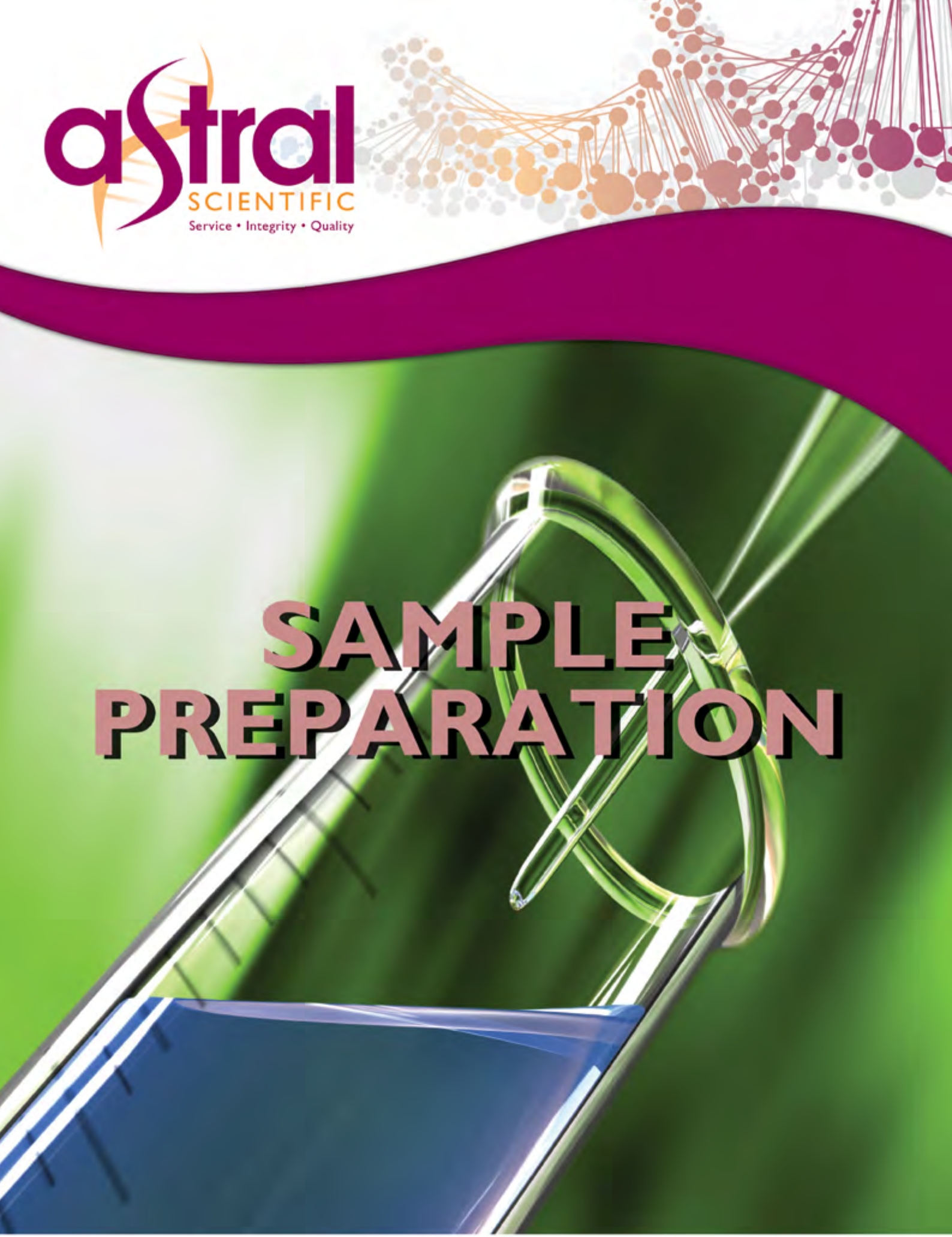

SAMPLE PREPARATION

Handbook and Selection Guide

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Cell or tissue lysis, fractionation and sample preparation are crucial tools for the purification, analysis and identification of proteins and their functions or roles. Unfortunately, there is no single procedure or protocol for optimal protein sample preparation as the techniques used are dependednt on numerous factors, including starting sample and downstream analysis techniques. There are generally three main stages:

1. Cell/Tissue Lysis: The release of proteins.
2. Protein Fractionation: The simplification of the protein complexity by fractionation.
3. Sample Preparation: The specific clean-up, concentration and additional treatments for subsequent analysis techniques (i.e. 1D or 2D protein electrophoresis).

Cell/ Tissue Lysis is the first step that is involved in cell extraction and protein purification. G-Biosciences offers a wide selection of protein extraction and lysis buffer systems. The range includes products that maintain biological activity of proteins, strong chaotropic extraction buffers that are 2D compatible and extraction systems for total proteomes.

Upon release of the proteins from the cell or tissue, simplification of the protein complex is performed. Protein analysis is often inhibited by the vast amount of proteins present and the large abundant proteins often inhibit the analysis of the low abundant proteins. Researchers overcome this problem by using fractionation, however inconsistencies in techniques and buffers often results in a lack of reproducibility. To aid in the simplification of samples, G-Biosciences offers several products for the rapid fractionation of proteins into multiple characteristics, including cellular location, hydrophobicity, post-translational modifications and other protein properties.

After lysis of the cell and protein fractionation has occurred, the final stage of identification of the protein, their roles and functions is to clean-up the sample for subsequent analysis techniques. G-Biosciences offers unique dialysis systems for the rapid removal of interfering agents from samples, ensuring no sample loss. Specialized clean-up kits are offered for protein samples destined for analysis by 1D and 2D electrophoresis. Several protein concentration kits are offered for the rapid concentration of dilute protein samples as well.

A wide range of lysis buffers and systems are available that offer researchers a large choice of lysis conditions, including total denaturing lysis, chaotropic extraction, gentle lysis for biologically active proteins, isolation of total proteomes and more.

CELL/TISSUE LYSIS

A wide selection of protein extraction and lysis buffer systems are offered. The range includes products that maintain biological activity of proteins (PE LB™ systems), strong chaotropic extraction buffers that are 2D compatible (2D-Xtract™, FOCUS™ Extraction Buffers) and extraction systems for total proteomes (FOCUS™ Proteome kits).

Common lysis buffers (RIPA), extraction tools (grinding resins), enzymes (lysozyme and Zymolyase®), protease and phosphatase inhibitors and other extraction accessories are also offered.

PROTEIN EXTRACTION & LYSIS BUFFER (PE LB™) SYSTEMS

Lysis and extraction of biologically active proteins from cellular and tissue samples is the first critical step for biochemical analysis. The correct selection of lysis and extraction buffers requires knowledge of the proteins of interest and the stability of their biological activities.

The Protein Extraction & Lysis Buffer (PE LB™) systems ensure good protein recovery, while maintaining the biological activity of the proteins. The solubilized proteins are suitable for enzyme assays, electrophoresis, folding studies, chromatographic studies and many other downstream applications.

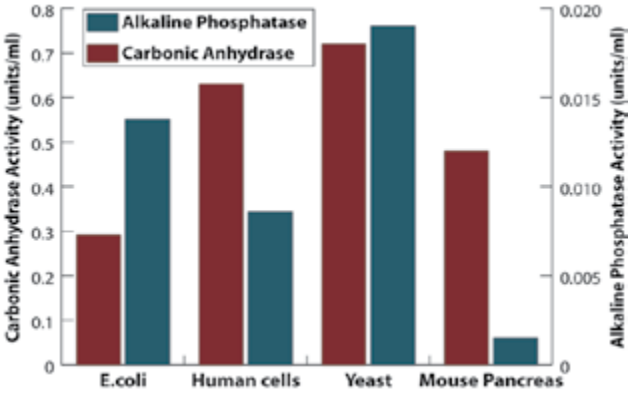

Figure 1: PE LB™ System maintains the biological activity of proteins. Extraction of carbonic anhydrase or alkaline phosphatase from E.coli, human cells, yeast and mouse pancreas with Bacterial, Mammalian Cell, Yeast and Tissue PE LB™ respectively. The resulting lysates were submitted to enzyme assays and both enzymes retain their biological activity.

The PE LB™ systems are based on a proprietary combination of organic buffering agents, mild non-ionic detergents, and a combination of various salts to enhance extraction of proteins and maintain stability of biological activities of the proteins.

Depending on application, additional agents such as chelating agents, reducing agents and protease and phosphatase inhibitors may be added to the PE LB™ buffer system.

The PE LB™ systems are compatible with most downstream applications including enzyme assays, running various chromatographic applications, gel electrophoresis applications, and protein folding procedures.

Bacterial PE LB™

Extraction of bacterial and recombinant proteins

For the extraction of biologically active soluble proteins, including recombinant proteins, and inclusion bodies from bacterial cells. A proprietary improvement on the lysozyme based lysis method, which allows for the extraction of soluble proteins and concurrent removal of nucleic acids (DNA & RNA) released during cell lysis. The Bacterial PE LB™ lysis eliminates viscosity build-up, allowing effective clarification with lower centrifugal forces.

Based on organic buffering agents and utilizes a mild non-ionic detergent, chelating agent, and a proprietary combination of various salts and agents to enhance extraction and stability of proteins. Depending on the required downstream application, additional agents such as reducing agents and protease inhibitors may be added. Bacterial PE LB™ has been tested for use with several widely used bacterial strains.

Supplied as a kit, which includes PE LB™ Lysozyme, a modified lysozyme preparation that contains nucleases and results in optimal lysis and minimal contamination. Bacterial PE LB™ buffer is also available separately for further downstream applications.

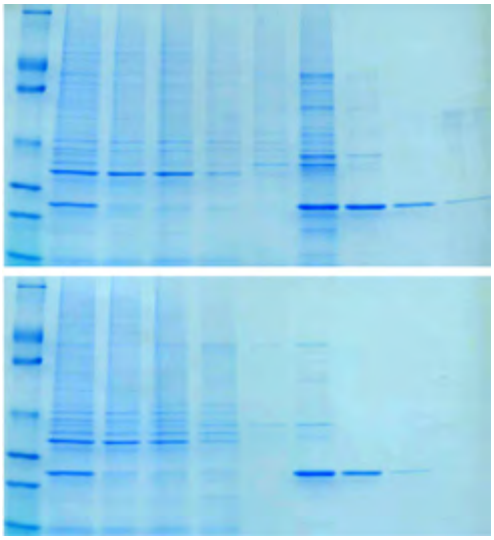

Figure 2: Bacteria expressing a His-tagged protein were lysed with Bacterial PE-LB™ and the recombinant protein was purified with HOOK™ 6X His Protein Purification kits (Top: Nickel resin; Bottom: Cobalt resin). Lane 1: PAGEmark™ protein ladder; 2: Cleared lysate; 3: Flow through; 4-6: Washes; 7-9: Elutions.

FEATURES

- Eliminates mechanical lysis and viscosity build-up
- Suitable for processing 100 x 50µl bacterial cell pellets

APPLICATIONS

- Lysis and extraction of proteins from bacterial cells
- For the isolation of biologically active proteins

CITED REFERENCES

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Cat. No.	Description	Size
786-176	Bacterial PE LB™ Kit including PE LB™ Lysozyme	100 preps
786-177	Bacterial PE LB™ buffer only	500ml

Yeast PE LB™

Developed for the extraction of biologically active, soluble proteins from yeast cells. Yeast PE LB™ is a proprietary improvement on the lyticase (Zymolyase®) based spheroplast preparation and extraction of soluble proteins from yeast cell method. Based on organic buffering agents and utilizes a mild non-ionic detergent, chelating agent, and a proprietary combination of various salts and agents to enhance extraction and stability of proteins.

A ready-to-use Zymolyase® preparation is also provided. Depending on the required downstream application, additional agents such as reducing agents and protease inhibitors may be added into Yeast PE LB™. Yeast PE LB™ has been tested on several widely used yeast strains. Suitable for processing 100 x 50µl yeast cell pellets. Yeast PE LB™ buffer is also available separately.

FEATURES

- Eliminates the need for glass bead lysis
- Supplied as a kit, containing Zymolyase®

APPLICATIONS

- Lysis and extraction of proteins from yeast cells
- Isolation of spheroplasts

CITED REFERENCES

Saribas, A.S., et al (2004) Glycobiology 14: 1217

Cat. No.	Description	Size
786-178	Yeast PE LB™ Kit including Zymolyase®	100 preps
786-179	Yeast PE LB™, buffer only	500ml

Mammalian Cell PE LB™

Mammalian Cell PE LB™ has been developed for extraction of total biologically active, soluble proteins from mammalian cultured cells. The Mammalian Cell PE LB™ is based on organic buffering agents and utilizes a mild non-ionic detergent, chelating agent, and a proprietary combination of various salts and agents to enhance extraction and stability of proteins. Depending on the required downstream application, additional agents such as reducing agents, phosphatase and protease inhibitors may be added into Mammalian Cell PE LB™. Mammalian Cell PE LB™ has been tested on a wide variety of mammalian cells and can be used for both suspension and adherent cells.

FEATURES

- Compatible with most enzyme assays including reporter gene assays (β-galactosidase, luciferase, chloramphenicol acetyltransferase), kinases (protein kinase C, protein kinase A, tyrosine kinase) & immunoassays (ELISA, Western blots, RIA)

APPLICATIONS

- For extraction of soluble proteins from adherent and suspension animal cultured cells
- Suitable for most applications including enzyme and protein purification applications, electrophoresis, Western blotting and 2D-gel analysis

CITED REFERENCES

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Yu, B. et al (2013) Life Sciences. 92:282
Eto, I. (2013) Metabolism. 62:873
More citations available at www.GBiosciences. com

Cat. No.	Description	Size
786-180	Mammalian Cell PE LB™	500ml

Tissue PE LB™

Developed for extraction of total biologically active, soluble proteins from animal tissues. Tissue PE LB™ is based on an organic buffer and utilizes a mild non-ionic detergent, chelating agent, and a proprietary combination of various salts and agents to enhance extraction and stability of proteins. Depending on the required downstream application, additional agents such as reducing agents and protease inhibitors may be added. Suitable for a wide variety of fresh and frozen animal tissues.

FEATURES

- Compatible with most enzyme assays including reporter gene assays (β-galactosidase, luciferase, chloramphenicol acetyltransferase), kinases (protein kinase C, protein kinase A, tyrosine kinase) & immunoassays (ELISA, Western blots, RIA)

APPLICATIONS

- Soluble protein extraction from fresh and frozen animal tissue
- Suitable for most applications including enzyme and protein purification applications, electrophoresis, Western blotting and 2D-gel analysis

CITED REFERENCES

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More citations available at www.GBiosciences. com

Cat. No.	Description	Size
786-181	Tissue PE LB™	500ml

Insect PE LB™

Insect PE LB™ has been developed for extraction of total biologically active, soluble proteins from adherent or suspension cultured insect cells, including Sf9 and Sf21. Insect PE LB™ utilizes a mild non-ionic detergent and a proprietary combination of various salts and agents to enhance extraction and stability of proteins. The Insect PE LB™ is fully compatible with downstream processes, such as electrophoresis and chromatography. Depending on the required downstream application, additional agents such as reducing agents and protease inhibitors may be added into Insect PE LB™.

FEATURES

- Provides a simple and versatile method for protein extraction from adherent or suspended Sf9 and Sf21 insect cells
- Compatible with electrophoresis and chromatographic applications

APPLICATIONS

- For extraction of soluble proteins from cultured insect cells
- Suitable for most applications including enzyme and protein purification applications, electrophoresis, Western blotting and 2D-gel analysis

Cat. No.	Description	Size
786-411	Insect PE LB™	250ml

DENATURING CHAOTROPIC EXTRACTION BUFFERS

2D-Xtract™

A protein solubilization buffer for 2D analysis must solubilize proteins effectively, without disturbing the native charge of the proteins. Urea based solubilization buffers solubilize proteins effectively, however can modify the native charge of the proteins, due to carbamylation. Urea exists in equilibrium with ammonium cyanate that modifies α- and ε-amino groups, inducing changes in the isoelectric point of proteins leading to artifactual results.

One way to minimize the risk of carbamylation is to prepare the urea based reagents fresh before each use. G-Biosciences developed 2D-Xtract™, a dry urea based pre-mixed and ready-to-use solubilization buffer. Simply add an appropriate volume of the supplied rehydration buffer to the dry buffer and then use to solubilize proteins, saving time and improving the quality of IEF/2D gel electrophoresis. 2D-Xtract™ has optimized concentrations of urea, thiourea, CHAPS and non detergent sulfobetaine (ND SB) 201. 2D-Xtract™ is also designed to be used as a rehydration buffer for IPG strips.

FEATURES

- Convenient and simple to use extraction buffer
- No preparation required, simply hydrate and use
- Prevents urea induced protein carbamylation
- Prevents waste of unused reagents

APPLICATIONS

- Suitable for sample extraction and solubilization for 2D gel electrophoresis and other applications
- Suitable for rehydration of IPG Strips

CITED REFERENCES

Powell, M.D. et al (2010) Proteomics. 4:337

Cat. No.	Description	Size
786-501	2D-Xtract™	For 50ml

FOCUS™ Protein Solubilization Buffer (PSB)

FOCUS™ Protein Solubilization Buffer is a dry, urea-based and ready-to-use buffer for protein solubilization. It is a proprietary modification of urea based chaotropic extraction buffers. Normal urea based buffers solubilize proteins effectively however can modify the native charge of the proteins, due to carbamylation, a process that modifies amino groups, inducing changes in the isoelectric point of proteins leading to artifactual 2D results.

PSB is a DRY, pre-mixed formulation of urea, thiourea, CHAPS and non detergent sulfobetaine (ND SB 201) for maximum solubilizing strength. The dry format allows researcher's to freshly rehydrate as much or as little as is required and therefore prevent urea induced carbamylation. Supplied with specific dilution buffer for optimal rehydration and protein solubilization conditions.

FEATURES

- Convenient and simple to use protein solubilization buffer
- Simply hydrate and use
- Prevents urea induced protein carbamylation
- Prevents waste of unused reagents

Cat. No.	Description	Size
786-PSB	FOCUS™ Protein Solubilization Buffer	For 50ml

FOCUS™ Extraction Buffers

Chaotropic extraction buffers that preserve the native charge of proteins

One of the most important considerations before running 2D gel electrophoresis is the choice of protein solubilization buffers. The suitable buffer must solubilize proteins effectively, without disturbing the native charge of the proteins. Urea, a common chaotrope, is widely used for solubilization and denaturation of proteins. One of the disadvantages of using urea is carbamylation. Urea in water exists in equilibrium with ammonium cyanate, the level of which increases with increasing temperature and pH. Cyanate reacts with α-amino and ε-amino groups of proteins and induces a change in the isoelectric point of proteins. This leads to artifactual results and therefore carbamylation must be avoided.

One way to minimize the risk of carbamylation is to prepare the urea based reagents fresh before each use. G-Biosciences has developed a series of dry urea based pre-mixed and ready-to-use solubilization buffers. Simply add an appropriate volume of the supplied rehydration buffer to the dry buffer and then use to solubilize proteins, saving time and improving the quality of IEF/2D gel electrophoresis.

FOCUS™ Extraction Buffers are also designed to be used as rehydration buffers for IPG strips.

FOCUS™ Extraction Buffers are experimentally optimized concentrations of critical agents, buffering and stabilizing agents, including urea, thiourea, Nonidet® P-40, CHAPS, and sulfobetaines (SB). The FOCUS™ Extraction Buffers are designed to produce optimal protein extraction and improved spot resolution for 2D gel analysis.

A range of FOCUS™ Extraction Buffers have been developed and depending on the nature of the samples, one or more of the buffers suitable for your applications can be ordered. FOCUS™ Extraction Buffer-I is suitable for most applications, however for stronger solubilization effects, we recommend FOCUS™ Extraction Buffer-II, -III, -IV, -V or -VI.

For analysis of a proteome, a single buffer may not be suitable and sequential solubilization using different FOCUS™ Extraction Buffers will help in identifying new proteins.

FEATURES

- Convenient and simple to use extraction buffers, simply hydrate and use
- Prevents urea induced protein carbamylation
- No artifactual protein bands due to dust and human skin contamination

APPLICATIONS

- Suitable for sample extraction and solubilization for 2D gel electrophoresis and other applications
- Suitable for IPG strip rehydration

	Major Components
FOCUS™ Extraction Buffer I	Urea & Nonidet® P-40
FOCUS™ Extraction Buffer II	Urea & CHAPS
FOCUS™ Extraction Buffer III	Urea, thiourea, CHAPS & ASB-16
FOCUS™ Extraction Buffer IV	Urea, thiourea, CHAPS & SB 3-10
FOCUS™ Extraction Buffer V	Urea, thiourea & CHAPS
FOCUS™ Extraction Buffer VI	Urea, thiourea, CHAPS & NDSB 201

Table 1: The major components of the FOCUS™ Extraction Buffers.

CITED REFERENCES

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Lee, D. and Chang, G. (2009) Meth. Mol. Biol. 536:23

Cat. No.	Description	Size
786-220	FOCUS™ Extraction Buffer I	For 50ml
786-221	FOCUS™ Extraction Buffer II	For 50ml
786-222	FOCUS™ Extraction Buffer III	For 50ml
786-223	FOCUS™ Extraction Buffer IV	For 50ml
786-219	FOCUS™ Extraction Buffer V	For 50ml
786-233	FOCUS™ Extraction Buffer VI	For 50ml
786-234	FOCUS™ Extraction Buffers I-VI Trial kit	For 10ml each buffer

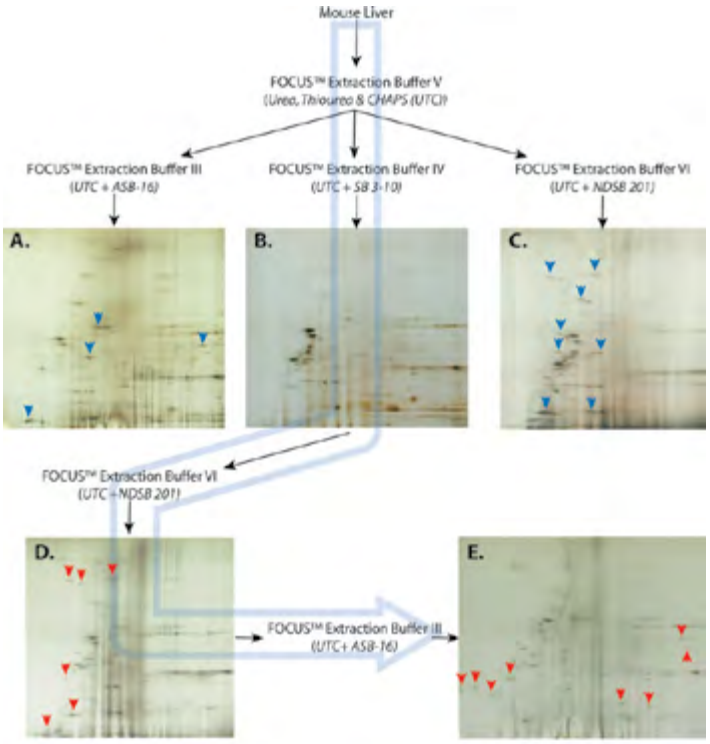

Figure 3: Serial Solubilization of Mouse Liver. Mouse liver was solubilized in FOCUS Extraction Buffer V and the insoluble material was further solubilized. The insoluble pellet was solubilized in either FOCUS™ Extraction Buffer III (A), IV (B) or VI (C) and the proteins were resolved by 2D electrophoresis. The blue arrowheads highlight a selection of different proteins compared to gel B. In a second analysis, the insoluble pellet from FOCUS™ Extraction Buffer V was serially extracted in FOCUS™ Extraction Buffer IV (B), then VI (D) and finally III (E). These were resolved by 2D electrophoresis. New proteins appearing at each stage are indicated with red arrows. UTC= Urea, thiourea & CHAPS.

TOTAL PROTEOME EXTRACTION

Isolate total proteomes from various species

An effective proteome analysis requires the preparation of a sample to bring the wide range of protein species into the dynamic range of detection. The absence of any standardized procedures for sample preparation has made proteome analysis extremely complicated, requiring a multitude of complicated skills, expensive equipment, and resources.

FOCUS™ Proteome Kits are for the preparation of total protein, including soluble, insoluble, membrane, cytoplasmic, nuclear, signal, phospho- and glyco-proteins. The FOCUS™ Proteome Kits are suitable for biological samples from tissues, cells, plants, yeast, bacteria and insects. These kits are simple to use, save time, improve the quality of protein analysis and enhance the chances of discovery of novel proteins. The kits are suitable for the analysis of proteins using electrophoresis and other biochemical techniques.

FOCUS™ Mammalian Proteome

Extracts and solubilizes nearly all of the proteins from mammalian samples, including membrane as well as soluble proteins, by a strong chaotropic extraction buffer to solubilize even the most difficult proteins. Suitable for biological samples from animal tissues and adherent and suspension cells.

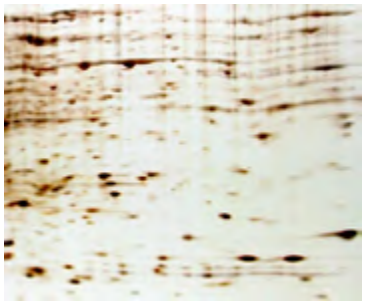

Figure 4: 2D electrophoresis gel of proteins isolated with FOCUS™ Mammalian Proteome from mouse liver.

FEATURES

- Single step extraction protocol
- Supplied with a strong, chaotropic extraction buffer
- For 50 x 100mg animal tissue or 50µl wet cell pellets

APPLICATIONS

- Extraction of total proteins from mammalian tissues and cells
- Suitable for sample preparation for 2D gel electrophoresis and other applications

Cat. No.	Description	Size
786-246	FOCUS™ Mammalian Proteome	50 preps

FOCUS™ Insect Proteome

Extracts and solubilizes nearly all of the proteins from insect cell cultures (i.e. Sf9 and Sf21), including membrane as well as soluble proteins, using a strong chaotropic extraction buffer to solubilize even the most difficult proteins.

FEATURES

- Single step extraction protocol
- For the extraction of protein from 50 x 50µl insect cell pellets

APPLICATIONS

- For 2D gel electrophoresis and other applications

Cat. No.	Description	Size
786-360	FOCUS™ Insect Proteome	50 preps

FOCUS™ Yeast Proteome

Specifically designed for yeast research and supplied with yeast specific reagents. Extracts and solubilizes nearly all of the proteins from yeast, including membrane and soluble proteins. Extraction is based on gentle lysis of yeast cells with a yeast lytic enzyme preparation, LongLife™ Zymolyase®, which has improved stability and shelf life. Enzymatic action is followed by extraction of total proteome with the supplied strong chaotropic extraction buffer that solubilizes even the most difficult proteins.

FEATURES

- Supplied with yeast specific lytic enzyme preparation and a strong proprietary chaotropic extraction buffer
- Suitable for 50 x 60µl yeast cell pellet preparations

APPLICATIONS

- Extraction of total proteins from yeast cells
- Suitable for sample preparation for 2D gel electrophoresis and other applications

Cat. No.	Description	Size
786-257	FOCUS™ Yeast Proteome	50 preps

FOCUS™ Bacterial Proteome

Specifically designed for bacterial research and supplied with bacteria specific reagents. Extracts and solubilizes nearly all of the proteins from E.coli, including membrane as well as soluble proteins. Extraction is based on the gentle lysis of bacterial cells with LongLife™ Lysozyme enzyme, followed by extraction of total proteome with the supplied strong chaotropic extraction buffer that solubilizes even the most difficult proteins. Our LongLife™ Lysozyme has improved stability and shelf life.

Figure 5: 2D electrophoresis gel of proteins isolated with FOCUS™ Bacterial Proteome from E.coli.

FEATURES

- Simple extraction protocol
- Supplied with bacterial specific lytic enzyme preparation
- Supplied with a strong, chaotropic extraction buffer
- Suitable for extraction from 50 x 50µl bacterial cell pellet

APPLICATIONS

- Extraction of total proteins from bacteria
- Suitable for sample preparation for 2D gel electrophoresis and other applications

Cat. No.	Description	Size
786-258	FOCUS™ Bacterial Proteome	50 preps

FOCUS™ Plant Proteome

Specifically designed for plant research and supplied with plant specific reagents, including reagents for removal of plant pigments and other natural products that may interfere with protein analysis. Extracts and solubilizes nearly all of the proteins from plants, including membrane as well as soluble proteins. Supplied with a strong proprietary chaotropic extraction buffer to solubilize even the most difficult proteins.

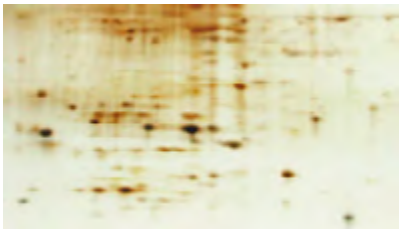

Figure 6: 2D electrophoresis gel of proteins isolated with FOCUS™ Plant Proteome from spinach leaves.

FEATURES

- Simple extraction protocol
- Supplied with reagents for removal of plant pigments and other natural products that may interfere with protein analysis
- Supplied with a proprietary chaotropic extraction buffer
- Extracts plant proteome from 25 x 0.5gm plant tissue preparations

APPLICATIONS

- Extraction of total proteins from plant tissues
- Suitable for sample preparation for 2D gel electrophoresis and other applications

Cat. No.	Description	Size
786-259	FOCUS™ Plant Proteome	25 preps

MISCELLANEOUS LYSIS PRODUCTS

Total Protein Extraction (TPE™)

For the extraction of total protein from cells & tissues for SDS-PAGE analysis

Universal lysis system for the solubilization of total proteins from animal, plant, yeast, bacteria, and other biological samples. Samples are ground in the buffer provided and then heated to solubilize the total protein.

The TPE™ kit provides a two component protocol that eliminates clump formation, protein loss, and other problems associated with total protein extraction procedures.

The TPE™ kit is based on optimized concentration of Tris and SDS and is suitable for running denaturing electrophoresis and other downstream applications.

FEATURES

- Ready-to-use buffers for extraction of total protein
- Two component extraction protocol
- Based on an optimized concentration of Tris and SDS
- Supplied with sufficient reagents for 50 x 250mg preparations

APPLICATIONS

- Suitable for solubilization of total proteins for electrophoresis and other applications

CITED REFERENCES

Liu, Y. et al (2011) Lipids Health Dis. 10:117
Prathyumnan, S. et al Int. J. Cur. Sci. Res. 3:120

Cat. No.	Description	Size
786-225	Total Protein Extraction (TPE™) Kit	50 preps

RIPA Lysis & Extraction Buffer

A complete lysis buffer for the release of cytoplasmic, membrane and nuclear proteins from adherent and suspension cultured mammalian cells. The RIPA lysis buffer is fully compatible with many applications, including reporter assays, protein assays, immunoassays and other protein purification techniques.

RIPA Lysis Buffer does not contain protease inhibitors, however it is fully compatible with our range of individual protease inhibitors and cocktails.

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More citations available at www.GBiosciences. com

Cat. No.	Description	Size
786-489	RIPA Lysis & Extraction Buffer	100ml
786-490	RIPA Lysis & Extraction Buffer	500ml

RAB (Reassembly) Buffer

A high salt RAB (Reassembly) buffer for the lysis of mammalian cells, including CHO[1,2], COS[3], NT2N[4,5] and HEK293[10]; *C. elegans*[6,10] and brain tissue[7-9].

Designed for the extraction of soluble proteins, does not extract detergent extractable insoluble proteins.

General procedure for sequential extraction of proteins:

1. Extract soluble proteins with RAB buffer
2. Extract detergent soluble proteins with RIPA Buffer
3. Extract detergent-insoluble material FA-solubilized protein with 70% formic acid.

CITED REFERENCES

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Cat. No.	Description	Size
786-91	RAB Buffer (Reassembly Buffer)	250ml

Gram-Negative Lysis Buffer

An extraction buffer for soluble proteins from Gram-negative bacteria. It is a proprietary improvement on the lysozyme based lysis in combination with various salts and agents, which allows extraction of soluble proteins. Depending on the application, additional agents such as reducing agents, chelating agent, nuclease and protease inhibitors cocktail may be added. This buffer has been tested for use with several widely used bacteria including E. coli strains

Cat. No.	Description	Size
786-566	Gram-Negative Lysis Buffer	125ml
786-567	Gram-Negative Lysis Buffer	250ml
786-579	Gram-Negative Lysis Buffer	500ml
786-580	Gram-Negative Lysis Buffer	1L

IBS™ Buffer

Inclusion bodies solubilization buffer

The expression of recombinant proteins is a popular and routinely used technique in protein studies. The expression of recombinant proteins often has one drawback and that is the recombinant proteins aggregate and form inclusion bodies, especially when expressed at high levels. The aggregated proteins are difficult to solubilize, due to the nature of aggregates, however we offer a selection of products for dealing with the range of issues involved with solubilizing and recovering active proteins from inclusion bodies.

The IBS™ buffer is specifically developed for solubilization of inclusion bodies.

Simple to use protocol as inclusion bodies are suspended in IBS™ Buffer, where they readily dissolve releasing the proteins of interest. Once the inclusion bodies are solubilized, the sample is ready for further analysis and other downstream applications.

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Cat. No.	Description	Size
786-183	IBS™ Buffer Kit	100ml

IBS-HP™ Buffer

Solubilization of hydrophobic proteins from inclusion bodies

We offer IBS-HP™ Buffer for the solubilization of inclusion bodies containing highly hydrophobic proteins. The IBS-HP™ Buffer contains optimized concentration of SDS, a buffering agent and urea. After solubilization of inclusion bodies, free SDS may be quenched or removed by competing with a non-ionic detergent. Supplied with optional DTT.

Cat. No.	Description	Size
786-183HP	IBS-HP™ Buffer Kit	100ml

PROTEASE INHIBITOR COCKTAILS

Individual protease inhibitors and protease inhibitor cocktails are available. These include broad range cocktails with >95% inhibition, species specific cocktails and cocktails for large sample volumes. For a complete selection of protease inhibitors and cocktails, download our Protease & Phosphatase Inhibitors, Enzyme & Assays Handbook.

ProteaseArrest™

A broad range protease inhibitor cocktail with wide species specificity

ProteaseArrest™ is a general protease inhibitor cocktail solution that is provided as a 100X concentrated, ready-to-use solution. The ProteaseArrest™ 100X solution format is suitable for small, analytical sample applications, as >95% inhibition is achieved by adding 10µl ProteaseArrest™ per ml sample. For samples with higher than normal protease levels, the volume of ProteaseArrest™ added can be increased for greater inhibition levels.

The cocktail contains reversible and irreversible inhibitors of serine, cysteine, calpain and metallo-proteases.

An optional EDTA solution is provided for enhanced metalloprotease inhibition. It is not present in the actual ProteaseArrest™ cocktail as it would inhibit the activity of proteins that require divalent cations (Ca^{2+}, Mg^{2+} or Mn^{2+}) for their biological activity. In addition, EDTA will inhibit the purification of proteins using immobilized metal affinity chromatography (IMAC), including 6X His tagged recombinant proteins.

Due to the optimized concentration of the various inhibitors, ProteaseArrest™ shows excellent inhibition of protease activities and is therefore suitable for the protection of proteins during preparation of samples and protein purification from animal tissues, plants, yeast and bacteria.

ProteaseArrest™ is also available as single use aliquots that are suitable for >95% protease inhibition in 10ml solutions. These *OneQuant*™ ProteaseArrest™ are provided for additional protease inhibitor cocktail convenience.

The ProteaseArrest™ format allows delivery of optimized concentrations of protease inhibitor, for example 2X or higher concentrations can be added for tissues with higher than normal protease concentrations; a feature not possible with tablet format protease inhibitor cocktails.

In our study, a 1X concentration of ProteaseArrest™ inhibits over 95% of protease activities (e.g. 0.5mg/ml mouse pancreas extract). The ProteaseArrest™ protease inhibitor cocktail demonstrated greater inhibition levels compared to similar protease inhibitor cocktails, including tablet formats. In independent studies, researchers have found that ProteaseArrest™ outperforms several leading manufacturer's protease inhibitor cocktails, including tablet formats, in the purification of plant proteins.

FEATURES

- Broad spectrum protease inhibitor cocktail
- 100X concentrated, ready-to-use solution
- High inhibition levels: 1X ProteaseArrest™ inhibits >95% of protease activities (i.e. 0.5 mg/ml mouse pancreas extract)

APPLICATIONS

- Inhibition of protease activity in protein preparations of mammalian, bacteria, plant, yeast and fungal lysates
- Protection of proteins from proteolysis in such applications as electrophoresis, purification, storage, assays, and other applications

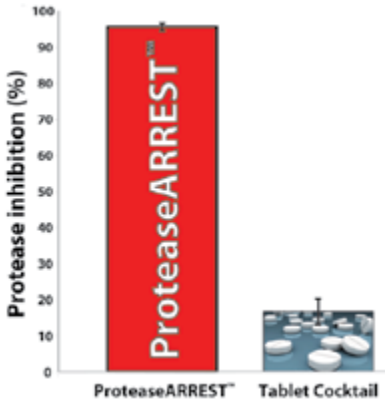

Figure 7: ProteaseArrest™ outperforms tablet format protease inhibitor cocktails. Protease inhibition in mouse pancreas lysate with EDTA-free ProteaseArrest™ and a commercially available EDTA-free tablet protease cocktail were compared using Protease Screening™ Kit. The assay used 0.5mg/ml pancreas lysate and incubation conditions of 37°C for 2.5 hours. ProteaseArrest™ inhibited over 95% of total proteases, 80% more compared to tablet inhibition.

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More citations available at www.GBiosciences. com

Cat. No.	Description	Size
786-108	ProteaseArrest™ [100X]	2ml
786-437	ProteaseArrest™ [100X]	5ml
786-329	*OneQuant*™ ProteaseArrest™ [100X]	24 x 100µl

PHOSPHATASE INHIBITOR COCKTAILS

PhosphataseArrest™ I

A broad spectrum phosphatase inhibitor cocktail consisting of five phosphatase inhibitors that target serine/threonine specific, tyrosine specific and dual specificity phosphatases.

PhosphataseArrest™ I is a stablized solution of sodium fluoride, sodium orthovanadate, sodium pyrophosphate, β-glycerophosphate & sodium molybdate.

Phosphatase Inhibitor	M.W.	Target Phosphatases
Sodium fluoride	42.0	Acid phosphatases
Sodium Orthovanadate	183.9	Tyrosine phosphatase, Alkaline phosphatase
Sodium Pyrophosphate	221.94	Serine/Threonine phosphatases
β-Glycerophosphate	306.1	Serine/Threonine phosphatases
Sodium Molybdate	205.92	Acid Phosphatase

PhosphataseArrest™ II

A phosphatase inhibitor cocktail consisting of five phosphatase inhibitors that target acid, alkaline and tyrosine phosphatases.

PhosphataseArrest™ II contains optimized concentrations of sodium fluoride, sodium tartrate, sodium orthovanadate, imidazole & sodium molybdate.

Phosphatase Inhibitor	M.W.	Target Phosphatases
Sodium fluoride	42.0	Acid phosphatases
Sodium Orthovanadate	183.9	Tyrosine phosphatases, Alkaline phosphatases
Sodium Tartrate	230.08	Acid phosphatases
Imidazole	68.08	Alkaline phosphatases
Sodium Molybdate	205.92	Acid Phosphatases

PhosphataseArrest™ III

A phosphatase inhibitor cocktail consisting of three phosphatase inhibitors, that target alkaline and serine/threonine phosphatases.

PhosphataseArrest™ III is a stable, convenient solution of cantharidin, *p*-bromotetramisole oxalate and calyculin.

Phosphatase Inhibitor	M.W.	Target Phosphatases
Cantharidin	196.2	Serine/Threonine phosphatases
p-Bromotetramisole Oxalate	373.23	Alkaline phosphatases
Calyculin	1009.17	Serine/Threonine phosphatases

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More citations available at www.GBiosciences. com

Selection Guide for Phosphatase Inhibitor Cocktails

Cat. No.	Description	Target Phosphatases	Size
786-450	PhosphataseArrest™ I [100X]	Serine/Threonine Tyrosine Dual Specificity	1ml
786-451	PhosphataseArrest™ II [100X]	Acid Alkaline Tyrosine	1ml
786-452	PhosphataseArrest™ III [100X]	Alkaline Serine/Threonine	1ml

Protein Solubilization Agents

G-Biosciences offers several proteomic grade chemicals and reagents to aid in protein solubilization.

Cat. #	Description	Size
786-279	Guanidine-HCL Solution (8M)	200ml
BC85	Guanidine-HCl	250g
BC88	Guanidine-HCl	500g
BC89	Urea	500g
C+/ .07	Dithiothreitol (DTT)	5g

Detergents

For a wide range of detergents, proteomic grade detergent solutions and detergent removal systems.

For full details of our Detergents, Proteases and Protease Inhibitor Systems, and Phosphatase Inhibitor Cocktails, go to our website and download the technical handbooks.

LongLife™ Enzyme Preparations

Enzymes regularly used in laboratory applications often require preparation of fresh solution before each use. Making fresh enzyme solution for each application is time consuming and wasteful. A wide variety of enzyme preparations in a ready-to-use format are offered.

LongLife™ enzyme preparations have a long shelf life and no weighing or buffer preparation is needed; simply take an aliquot and add to your sample. LongLife™ enzyme preparations contain cofactors necessary for optimal enzymatic activity. Supplied in suspension form and when stored properly have a one year shelf life.

ENZYMES OFFERED

- LongLife™ Zymolyase®: digestion of yeast & fungal cell walls.
- LongLife™ Lysozyme for the digestion of bacterial cell walls.
- LongLife™ PE LB Lysozyme for the digestion of bacterial cell walls and nucleic acids. Fully compatible with the PE LB buffer system. Reduces viscosity build-up due to presence of nucleases.
- LongLife™ Proteinase K for the digestion of proteins in nucleic acid preparations.
- LongLife™ Nuclease for the removal of nucleic acids.
- LongLife™ RNase for the digestion of RNA.
- LongLife™ DNase for the digestion of DNA.

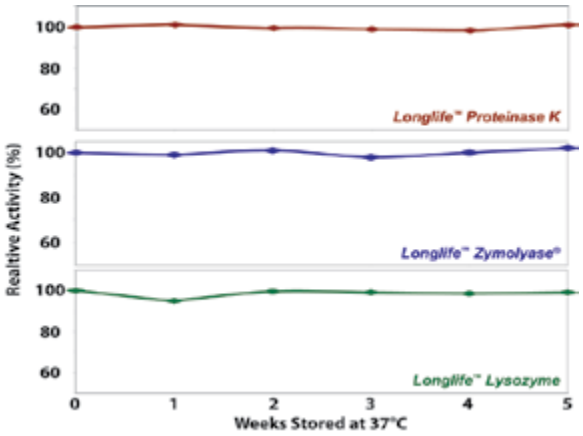

Figure 8: LongLife™ Enzymes are highly stable. Each enzyme preparation was tested over a period of 4 weeks at 37°C: and compared with LongLife™ enzyme preparations stored at -20°C.

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Cat. No.	Description	Size
786-036	*LongLife*™ Zymolyase® [1.5U/µl]	2 x 0.5ml
786-037	*LongLife*™ Lysozyme [1,500U/µl]	2 x 0.5ml
786-042	*LongLife*™ PE LB Lysozyme [1,500U/µl]	2 x 0.5ml
786-038	*LongLife*™ Proteinase K [5mg/ml]	2 x 0.5ml
786-039	*LongLife*™ Nuclease [10U/µl]	2 x 0.5ml
786-040	*LongLife*™ RNase [10U/µl]	2 x 0.5ml
786-041	*LongLife*™ DNase	0.5ml

GRINDING TOOLS

EZ-Grind™

A highly efficient grinding resin that is aliquoted into 1.5ml grinding tubes and supplied with matching pestles. The resin is for optimal grinding of biological samples for the extraction of both proteins and DNA. The resin is a neutral abrasive material that does not bind proteins or nucleic acids.

The combination of resin, pestles and tubes allows for efficient disruption of tissues, cells, nuclei and other cellular organelles.

Figure 9: EZ-Grind™ pestles and tubes with resin.

FEATURES

- Disrupts small tissue and cell samples for protein extraction
- 1.5ml tubes, grinding resin, and disposable pestles
- Process up to 100mg of sample per tube in about 10 min

CITED REFERENCES

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Cat. No.	Description	Size
786-139	EZ-Grind™	20

Molecular Grinding Resin™

Ideal for grinding small samples and the subsequent preparation of proteins and nucleic acids. The resin consists of high tensile micro particles, which effectively disrupt nuclei and other cellular organelles. The resin is fully compatible with any homogenization technique, including high speed mechanical grinders and sonicators.

Molecular Grinding Resin™ does not bind proteins or nucleic acids, minimizing loss. Simply mix the Molecular Grinding Resin™ with the biological samples and grind or homogenize the sample.

Supplied with enough resin for 200 isolations from 100mg tissue. For added convenience, Molecular Grinding Resin™ is also supplied with matching pestles and 1.5ml tubes.

FEATURES

- High tensile micro particles for grinding small samples

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Cat. No.	Description	Size
786-138	Molecular Grinding Resin™	5 x 1ml
786-138PR	Molecular Grinding Resin™ with pestles & tubes	5 x 1ml & 100 pestles & tubes
786-138P	Pestles & Tubes	100 pestles & tubes

Affinity Purification Resins

The analysis of a proteome is often inhibited by the vast amount of proteins, with large abundant proteins inhibiting the signal of lower abundance and often more interesting proteins. Researchers overcome this problem by using fractionation, however inconsistencies in techniques and buffers often results in a lack of reproducibility.

G-Biosciences offers a wide selection of fractionation kits for processing samples from cells, tissues, bacteria, yeast, plants, and other types of samples. A selection of sample preparation accessories and supplies are also included. The following kits, accessories, and supplies are suitable for analysis of proteins using electrophoresis and other biochemical techniques.

The FOCUS™ line of products allow for the fractionation of a large selection of biological samples into a multitude of different fractions and these resulting fractions are compatible with 2D electrophoresis and subsequent protein identification techniques.

FOCUS™ Soluble & Insoluble

A complete kit for the selective preparation of soluble (hydrophilic) and insoluble (hydrophobic) proteins from mammalian tissues and cells, plants, yeast, bacteria, and other biological samples. The kit comes with reagents necessary for fractionation of soluble and insoluble fractions, including a strong chaotropic extraction buffer to solubilize difficult proteins.

The FOCUS™ Soluble & Insoluble kit is supplied with a specific clean-up kit for the preparation of each fraction for isoelectric focusing and 2D electrophoresis for improved spot resolution.

FOCUS™ Soluble & Insoluble kit is designed for 50 preps, where one prep is:

- 100mg mammalian tissue
- 50µl wet animal cell pellet
- 50µl wet yeast pellet
- 50µl wet bacteria pellet
- 250mg plant tissue

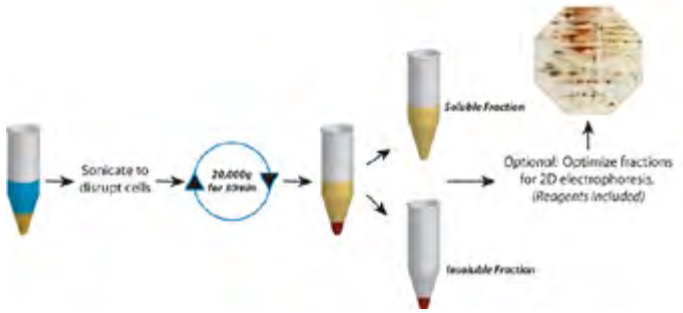

Figure 10: Scheme for FOCUS™ Soluble & Insoluble Kit.

FEATURES

- Generates soluble and insoluble fractions
- Fractions fully compatible with 2D electrophoresis

APPLICATIONS

- Extraction of soluble and insoluble proteins from tissues, cells, plants, yeast, bacteria and other sources
- Suitable for wide range of downstream applications, including 1D & 2D electrophoresis, Western blotting and mass spectrometry

Cat. No.	Description	Size
786-247	FOCUS™ Soluble & Insoluble Kit	50 Preps

Nuclear & Cytoplasmic Extraction

The Nuclear & Cytoplasmic Extraction Kit is for the enrichment of cytoplasmic and nuclear fractions from cultured cells and tissues. The kit generates a cleaner separation of cytoplasmic proteins from nuclear proteins and is ideal for expression and transport studies.

This kit is based on organic buffers and contains a proprietary combination of various salts and agents. The kit is provided with reagents for solubilization of nuclear fraction.

This kit is for >50 preps, depending on the tissue used, where one prep is $20x10^6$ mammalian cells or 100mg mammalian tissue.

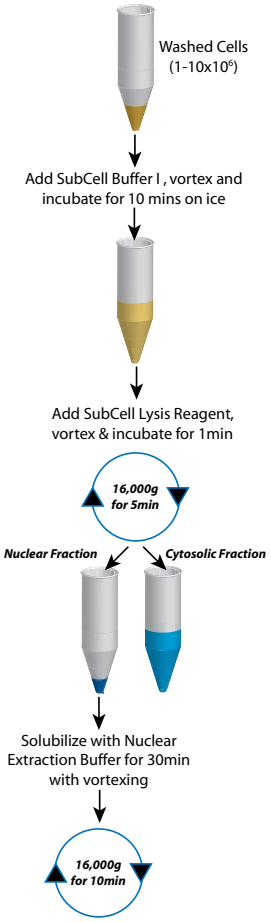

Figure 11: Nuclear & Cytoplasmic Extraction Kit scheme.

FEATURES

- For generation of cytosolic and ultra pure nuclear fractions
- Provided with nuclear protein solubilization buffers

APPLICATIONS

- Extraction of cytosolic and nuclear protein fractions for transport studies, electrophoresis and other applications

Cat. No.	Description	Size
786-182	Nuclear & Cytoplasmic Extraction Kit	100 Preps

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FOCUS™ Cytoplasmic & Nuclear Proteins

Fractionation of cytoplasmic and nuclear proteins from cells and tissues

Supplied with a strong chaotropic extraction buffer to solubilize both cytoplasmic and nuclear proteins, which is fully compatible with 2D gel electrophoresis.

FOCUS™ Cytoplasmic & Nuclear proteins fractionation kit is based on the Nuclear & Cytoplasmic Extraction Kit, however it has been modified to be fully compatible with 2D electrophoresis and subsequent downstream processes.

The kit is provided with reagents necessary for fractionation of cytoplasmic and nuclear proteins as well as solubilization buffer suitable for IEF/2D gels for better spot resolution.

This kit is designed for >50 preps, depending on the tissue used, where one prep is $20x10^6$ mammalian cells or 100mg mammalian tissue.

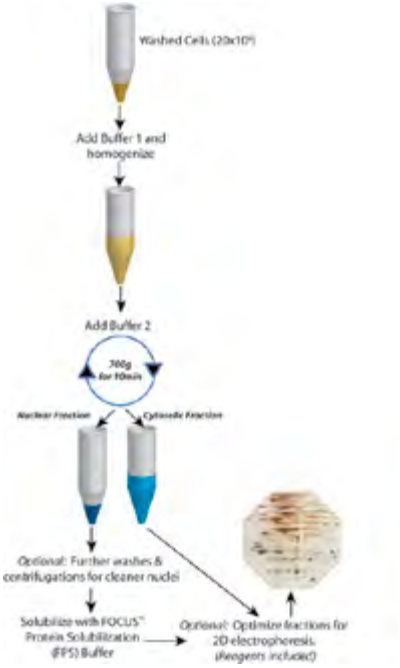

Figure 12: FOCUS™ Cytoplasmic & Nuclear Proteins scheme.

FEATURES

- Includes chaotropic extraction buffer for solubilization of nuclear and cytoplasmic proteins
- Fully compatible with 2D analysis

APPLICATIONS

- Fractionation of nuclear and cytoplasmic proteins from cells and tissues
- Suitable for wide range of downstream applications, including 1D & 2D electrophoresis, Western blotting and mass spectrometry

Cat. No.	Description	Size
786-248	FOCUS™ Cytoplasmic & Nuclear Proteins	50 Preps

CITED REFERENCES

Kim, Y.H. et al (2007) Neurobiol. Dis. 26:569
Wang, T. et al (2007) Biochem. Bioph. Res. Co. 352:203
Kim, Y.H. et al (2007) Life Sciences. 81:1167
Rehman, A. et al (2005) Breast cancer Res. 7:R765

FOCUS™ Membrane Proteins

FOCUS™ Membrane Proteins is a rapid and highly reproducible method for preparation of membrane or hydrophobic proteins from biological samples for 2D-gel analysis or other applications. Membrane proteins are extracted with a single step phase partition, with an efficiency greater than 90% with minimal contamination from hydrophilic proteins.

The kit is provided with reagents necessary for extraction of membrane proteins and their subsequent preparation for isoelectric focusing or 2D gel resolution for improved spot resolution.

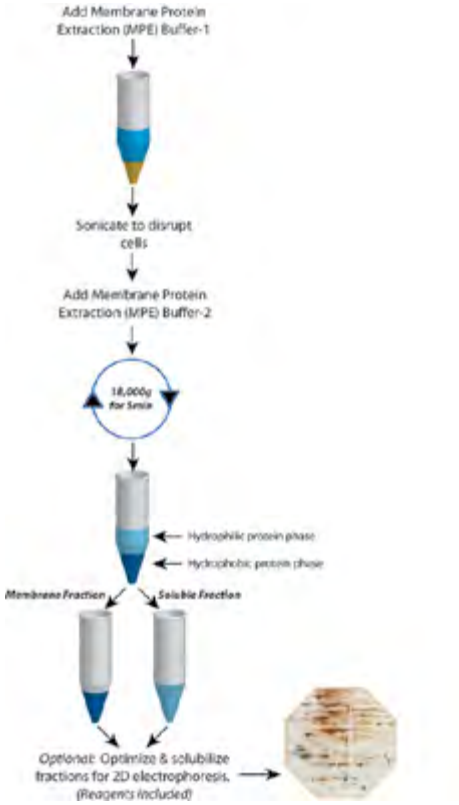

Figure 13: FOCUS™ Membrane Proteins scheme.

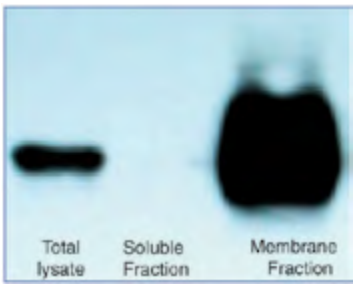

Figure 14: Mouse liver was processed with FOCUS™ Membrane Proteins kit. Enriched membrane and soluble fractions were resolved by SDS-PAGE, transferred and probed with antibodies against caveolin.

FEATURES

- Phase partition based extraction of membrane proteins
- Fractions suitable for 2D electrophoresis and other applications

APPLICATIONS

- Selective fractionation of membrane proteins from tissues, cells, plants, yeast, bacteria, insects, and other sources
- Suitable for membrane protein preparation for a wide range of downstream applications, including 1D & 2D electrophoresis, Western blotting and mass spectrometry

Cat. No.	Description	Size
786-249	FOCUS™ Membrane Proteins	50 Preps

FOCUS™ SubCell Kit

FOCUS™ SubCell kit enables the fast and easy isolation of nuclear, enriched mitochondrial, membrane and cytosolic fractions from animal cells and tissue. FOCUS™ SubCell is suitable for cultured animal cells and is adaptable for animal tissues.

FOCUS™ SubCell kit was used to evaluated the fractionation of NIH3T3 cells into fractions enriched in nuclear, mitochondria, cytosolic membrane or soluble proteins. After separation, the fractions were probed by Western blot with fraction specific antibodies and the mitochondrial fractions were analyzed for intact outer and inner membrane and mitochondrial functionality.

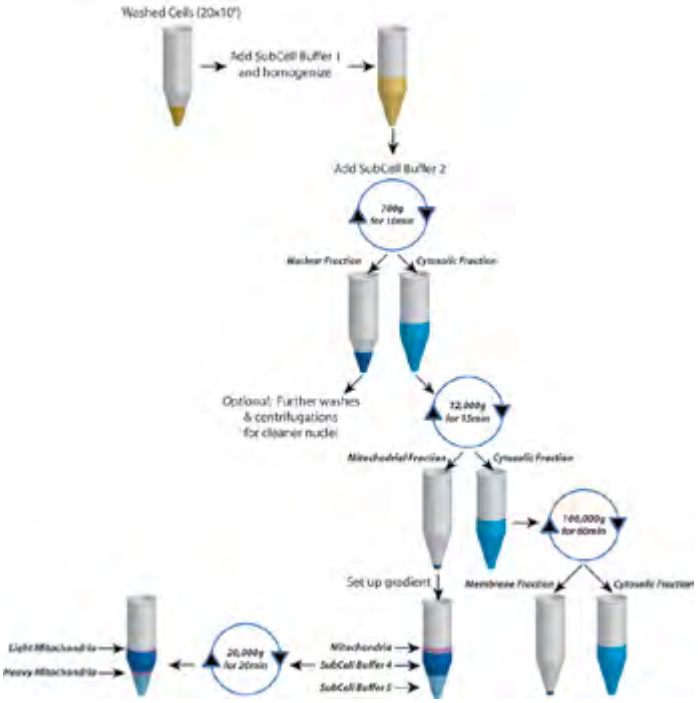

Figure 15: FOCUS™ SubCell Kit scheme.

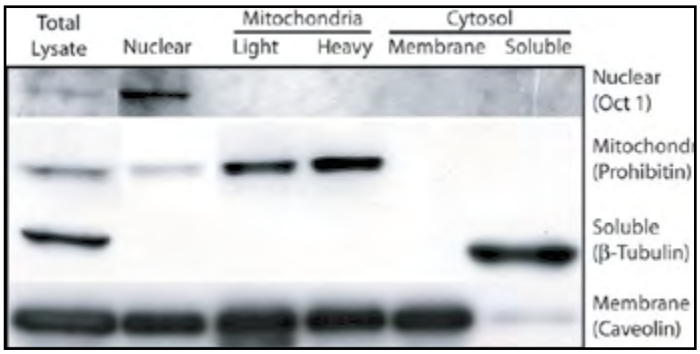

Figure 16: FOCUS™ SubCell fractionation of NIH3T3 cells. 4% total lysate and nuclear fraction and 15% heavy and light mitochondria and cytosolic membrane and soluble protein fractions were resolved on a 4-20% SDS-polyacrylamide gel. The proteins were transferred to a PVDF membrane, which was probed with antibodies specific for Oct-1, a nuclear transcription factor; Prohibitin, a mitochondrial marker; β-tubulin, a cytosolic protein and caveolin, a membrane associated protein.

The result of the immunoblot, using antibodies for specific cellular compartments. Oct-1, a nuclear transcription factor, was detected as a major band in the enriched nuclear fraction and a weaker band of the same molecular weight in the total lysate. The nuclear protein was not visualized in the mitochondrial of cytosolic fractions. Prohibitin is an evolutionarily conserved protein located in the inner membrane of mitochondria and was strongly localized to both the heavy and light mitochondrial fractions. A small amount of prohibitin localized to the nuclear fraction, however several researchers have reported that prohibitin can have a nuclear location (3, 4). The distribution of soluble cytosolic proteins was determined with antibodies against β-tubulin and this was localized solely to the total lysate and the soluble cytosolic fraction. Finally, the distribution of the cellular membranes was detected by caveolin. Caveolin was found in all fractions except the soluble cytosolic fraction. Recent literature suggests that the location of caveolin is not restricted to the caveolae on cytosolic membranes, but can be found involved with the secretory pathway and mitochondria and can be localized to all intracellular membranes (5, 6). This data combined with the other immunoblot data demonstrates that the FOCUS™ SubCell kit can successfully fractionate cells into enriched nuclear, light and heavy mitochondrial and cytosolic membrane and soluble protein fractions. The cytochrome C oxidase assay was used to determine the percentage of undamaged mitochondria in the enriched heavy and light mitochondrial fractions. The heavy fraction consisted of 85% and the light fraction 79% undamaged, intact mitochondria. To ensure the enriched mitochondrial fractions were active, the JC-1 assay was used. The enriched heavy and light mitochondrial fraction retain the integrity of their inner membranes and their activity.

The FOCUS™ SubCell kit is fast and convenient for the generation of enriched nuclear, mitochondrial (heavy and light), membrane and soluble cytosolic fractions. The enriched mitochondrial fraction contains a high percentage of intact mitochondria, which retain their activity.

FEATURES

- Fractions suitable for wide range of downstream applications, including 1D & 2D electrophoresis and Western blotting
- Isolated mitochondria are ≥90% active

APPLICATIONS

- For mitochondrial, nuclear, cytosolic and membrane fractions

CITED REFERENCES

Dagda, R.K. et al (2014) J. Neurochem. 128:864
Dodmane, P.R. et al (2014) Toxicol. Sci. 137:36
Stallings, N.R. et al (2013) PLOS. DOI: 10.1371/journal.pone.0071793
Melendez, J. et al (2013) Gastroenterology. 145:808
Zhu, J.H. et al (2012) Cell Death Dis. 3:e312
Losel, R.M. et al (2010) PLOS. DOI: 10.1371/journal.pone.0009713
Stallings, N.R. et al (2010) Neurobiol. Diss 40:404
Titsworth, W. et al (2009) Glia. 57:1521
Upreti, M. et al (2008) Mol Cancer Ther 7:2224
Son, M. et al (2007) PNAS 104:6072
Maiuri, M.C. et al (2007) EMBO J. 26:2527

Cat. No.	Description	Size
786-260	FOCUS™ SubCell Kit	50 Preps

Cat. No.	Description	Size
786-915	Silica Magnetic Beads	5ml resin
786-916	Silica Magnetic Beads	25ml resin
786-917	Silica Magnetic Beads	100ml resin

FOCUS™ Mitochondria

Specifically designed for the isolation of intact mitochondria from cultured mammalian cells. This kit allows for the fast and efficient fractionation of the cytoplasm of cultured mammalian cells into an enriched fraction of mitochondria. The majority (>90%) of the mitochondria have intact inner and outer membranes and therefore retain their functionality.

This kit is highly adaptable for use with animal tissues and other sources of mitochondria. The kit is designed for 50 preps, where one prep is equivalent to $2x10^7$ mammalian cells or 100mg mammalian tissue.

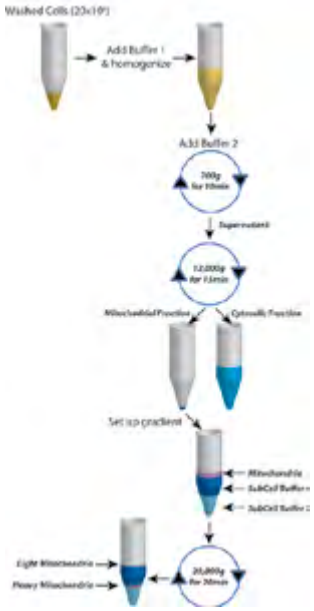

Figure 17: FOCUS™ Mitochondria scheme.

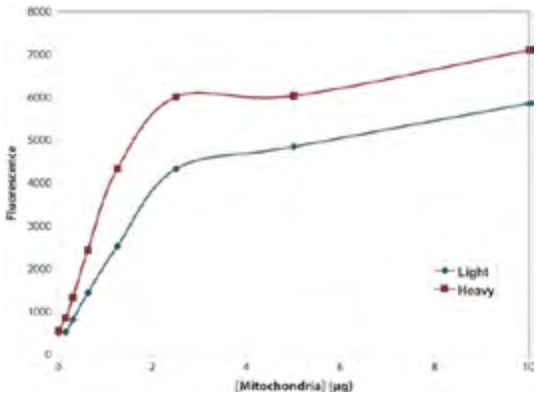

Figure 18: Activity of Mitochondria as determined with a JC-1 Assay.

FEATURES

- Fast and efficient fractionation of active heavy & light mitochondria
- Suitable for cultured animal cells and tissues

APPLICATIONS

- Fraction of mammalian cells and tissues into a mitochondrial rich fraction
- Suitable for wide range of downstream applications, including 1D & 2D electrophoresis, Western blotting and mass spectrometry

Cat. No.	Description	Size
786-022	FOCUS™ Mitochondria Kit	50 Preps

CITED REFERENCES

Dagda, R.K. et al (2014) J. Neurochem. 128:864
Rigobello, M.P. et al (2009) Free Rad. Biol. Med. 47:710
Wang, T. et al (2007) Biochem. Bioph. Res. Co. 352:203

FOCUS™ Global Fractionation

FOCUS™ Global Fractionation kit is designed to fractionate complex biological samples into cytosolic and membrane fractions. The resulting membrane fractions are subsequently fractionated into either peripheral and integral membrane proteins or lipid raft associated proteins and detergent soluble membrane proteins.

Lipid rafts are membrane microdomains that are enriched in caveolin, cholesterol, glycolipids, sphingolipids and glycosyl-phosphatidylinositol. Lipid rafts are also known as detergent-insoluble glycolipid-enriched complexes (GEMs) or DIGs. Many signaling proteins, including glycosylphosphatidylinositol (GPI)-anchored proteins, doubly-acylated tyrosine kinases of the Src family, and transmembrane proteins, are located in lipid rafts. Lipid raft localized proteins have been shown to be involved in intracellular membrane trafficking and signaling.

FOCUS™ Global Fractionation is designed for >50 preps, where one prep is:

- 100mg mammalian tissue
- 50µl wet animal cell pellet
- 50µl wet yeast pellet
- 50µl wet bacteria pellet
- 250mg plant tissue

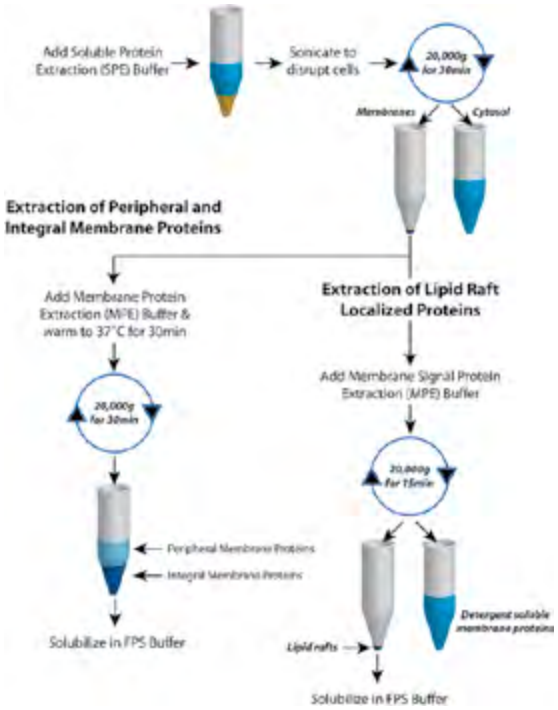

Figure 19: FOCUS™ Global Fractionation scheme.

FEATURES

- For integral, peripheral & lipid raft associated protein fractions
- Fractionation of complex proteomes into multiple fractions

APPLICATIONS

- Membrane proteins from tissues, cells, plants, yeast, bacteria, insects and other sources
- Fractionation of membrane proteins from lipid rafts
- For downstream applications, including 1D & 2D electrophoresis, Western blotting and mass spectrometry
- Suitable for proteomics and cell signaling studies

Cat. No.	Description	Size
786-018	FOCUS™ Global Fractionation	50 preps

CITED REFERENCES

Zhao, S. et al (2014) J. Proteomicss. 101:102
Wang, J. et al (2013) Appl. Microniol. Biotech. 97:2077

Fraction-FOCUS™

2D electrophoresis and mass spectrometry is routinely used for identification of novel proteins, however the greatest challenge in protein identification is achieving suitable resolution of proteins. The high dynamic range of a species proteome means that the more abundant proteins mask the less abundant and often more interesting proteins.

Fractionation simplifies the protein composition and allows for improved resolution and simplified 2D maps, which in turn allows for improved analysis and interpretation and greatly increases the chances of identifying novel and less abundant proteins.

Fraction-FOCUS™ utilizes proven technology to fractionate and concentrate all proteomes into multiple fractions; simplifying 2D maps and enhancing detection of low abundant proteins. Fraction-FOCUS™ is fully compatible with all downstream protein identification techniques.

There is no detectable loss of material total protein during the Fraction-FOCUS™ procedure. At the end of the fractionation, cellular proteins are in one of many fractions. The entire fractionation is carried out in micro scale.

The Fraction-FOCUS™ kit is designed for 10 preparations, where one preparation is:

- 100mg mammalian tissue
- 50µl wet animal cell pellet
- 50µl wet yeast pellet
- 50µl wet bacteria pellet
- 250mg plant tissue

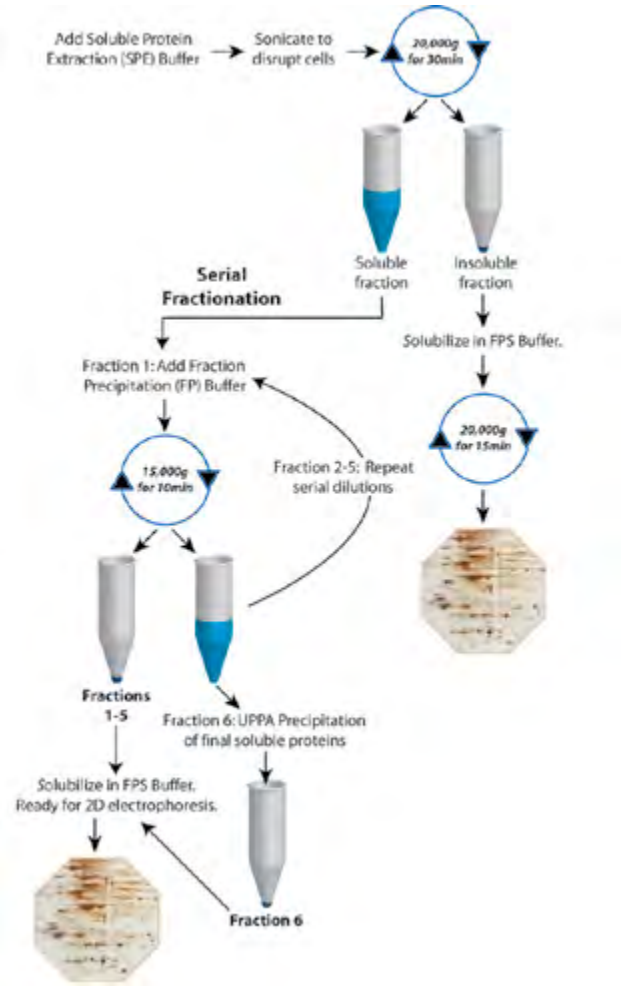

Figure 20: Scheme for Fraction-FOCUS™.

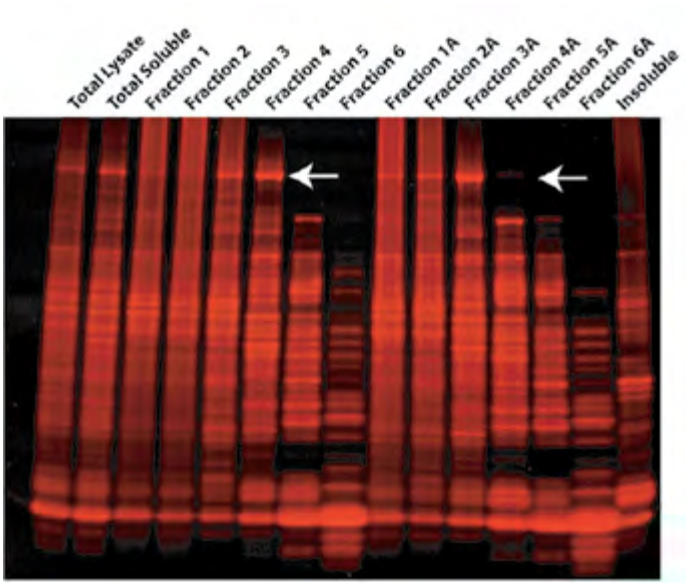

Figure 21: Mouse liver fractionated with Fraction-FOCUS™. Mouse liver lysate was analyzed with Fraction-FOCUS™ (Fractions1-6, normal protocol; Fractions 1A-6A, alternative protocol). 5µg protein was resolved by SDS-PAGE and stained with a fluorescent stain. Fraction-FOCUS™ simplifies the protein maps, compared to total lysate, and a simple change in the protocol generates a different protein profile and allows proteins to be concentrated in the same fractions (see arrows).

FEATURES

- Serial fractionation of protein samples into multiple protein fractions
- Resulting fractions are fully compatible with 2D electrophoresis or isoelectric focusing and other applications

APPLICATIONS

- Fractionation of soluble proteins for cleaner 2D gels maps
- Suitable for electrophoresis and other applications

Cat. No.	Description	Size
786-168	*Fraction*-FOCUS™	10 Preps

FOCUS™ Glycoprotein

Glycoproteins are proteins that are post-translationally modified by the addition of carbohydrates. The carbohydrates are coupled to asparagine (N-linked) and serine/ threonine (O-linked) residues during passage through the endoplasmic reticulum and golgi apparatus. They are commonly found decorating the cell membrane with the carbohydrate moieties in the extracellular space. Glycosylated proteins play critical roles in cell signaling, inflammation, cell-to-cell adhesion and in the immune response.

FOCUS™ Glycoprotein rapidly fractionates glycoproteins that have terminal a-D-mannosyl and a-D glycosyl residues. FOCUS™ Glycoprotein utilizes spin columns containing the immobilized lectin Concanavalin A for rapid glycoprotein isolation.

FOCUS™ Glycoprotein kit was evaluated in the fractionation of Jurkat cells. After fractionation, fractions were analyzed by 1D electrophoresis stained with Reversible Zinc Stain™ and 2D electrophoresis stained with a fluorescent protein stain.

Shown below is the protein profile of Jurkat cells treated with FOCUS™ Glycoprotein. As expected the majority of the Jurkat cell proteome is removed in the flow through and washing steps as only a small percentage of the proteome is glycosylated. A large number of glycosylated proteins are isolated from the FOCUS™ Glycoprotein columns and the protein profiles change when each Glyco-Elution Buffer is used.

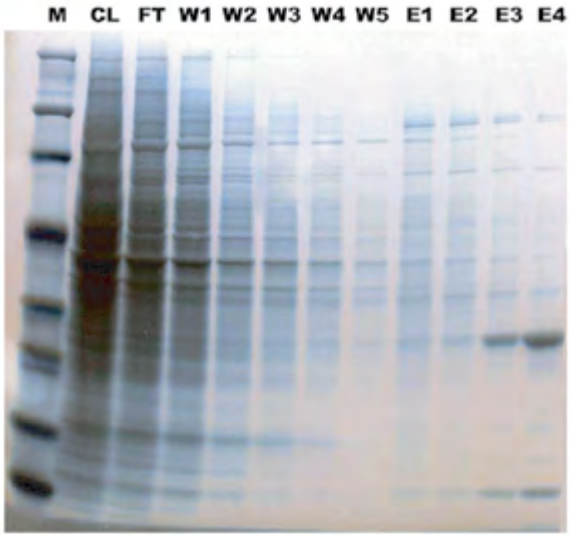

Figure 22: FOCUS™ Glycoprotein isolates multiple glycoproteins. Jurkat cells were lysed by sonication, centrifuged and the supernant (CL) loaded onto a FOCUS™ Glycoprotein column. The column was centrifuged and the flow through (FT) collected. The column was washed (W1-5) and the glycoproteins were eluted with Glyco Elution Buffer I (E1-2), Glyco Elution Buffer II (E3) and then Glyco Elution Buffer III (E4). 10µl was loaded onto a SDS-PAGE gel, the proteins were resolved and visualized with Reversible Zinc Stain™.

In addition, the low abundant proteins are now easily visualized in the elution fractions as the majority of the proteome has been removed and due to the high sensitivity of the Reversible Zinc Stain™.

A comparison of the crude Jurkat cell lysate and an equal mix of the elutions E1, E2 and E3 are shown. Firstly, the use of the FOCUS™ Glycoprotein kit significantly reduces the complexity of the 2D map, making it easy to identify and isolate glycoprotein protein spots. Secondly, the concentration of the proteins is stronger due to the enrichment of the glycoproteins by the FOCUS™ Glycoprotein kit.

FOCUS™ Glycoprotein is ideal for the fractionation, enrichment and isolation of glycoproteins from a wide selection of samples, including tissues, cell lysates and serum. The fast and convenient spin column format bind and immobilize ~5mg glycoproteins and the enriched, eluted glycoproteins are ready for further analysis within 90 minutes.

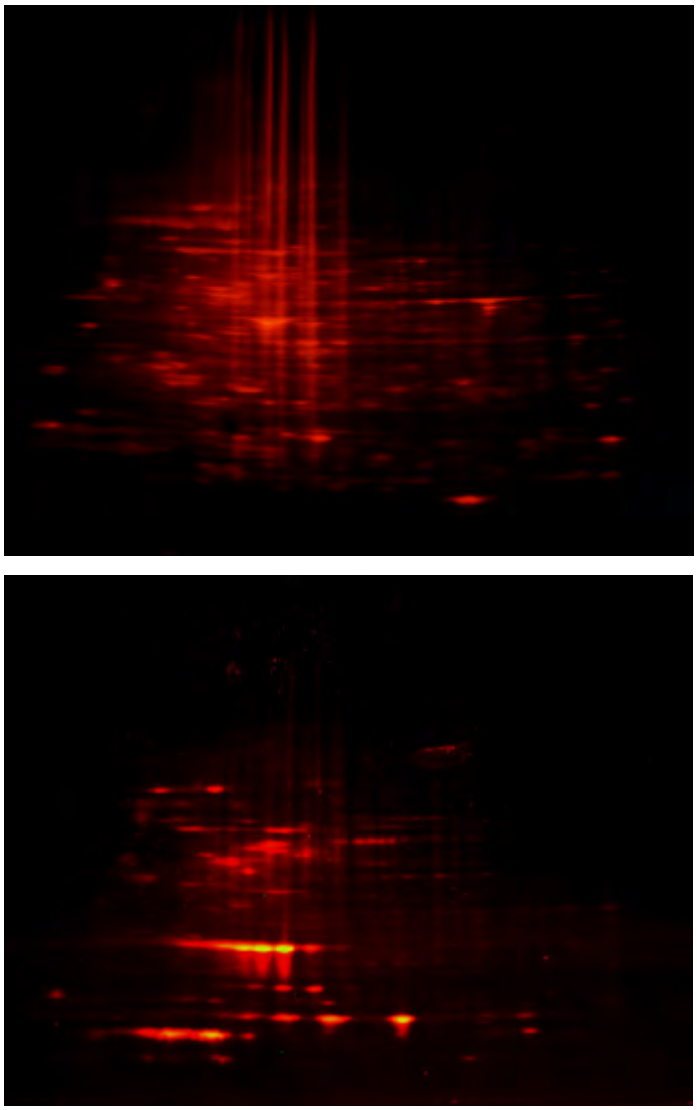

Figure 23: Comparison of crude Jurkat cell lysate (A) and glycoproteins (B) isolated with FOCUS™ Glycoprotein. Glycoproteins were isolated from Jurkat cells using FOCUS™ Glycoprotein kit as described in the Methods section. The first 3 elution fractions were combined and the eluents and a sample of the crude Jurkat cell lysate were treated with Perfect-FOCUS™ to prepare them for 2D electrophoresis. The first dimension was run on 11cm pH3-10 strips and the second dimension on 4-20% SDS polyacrylamide gels. Proteins were visualized with a fluorescent protein stain.

FEATURES

- Spin column protocol
- Uses a high capacity lectin binding resin (10-20mg/ml resin)
- Elution of glycoproteins within 90 minutes with a set of three rapid elution buffers

APPLICATIONS

- Fractionation and enrichment of glycoprotein
- Suitable for wide range of downstream applications, including 1D & 2D electrophoresis, Western blotting and mass spectrometry

Cat. No.	Description	Size
786-253	FOCUS™ Glycoprotein	10 Preps

FOCUS™ Signal Proteins

Lipid rafts are membrane microdomains that are enriched in caveolin, cholesterol, glycolipids, sphingolipids and glycosyl-phosphatidylinositol. Lipid rafts are also known as detergent-insoluble glycolipid-enriched complexes (GEMs) or DIGs. Many signaling proteins, including glycosylphosphatidylinositol (GPI)-anchored proteins, doubly-acylated tyrosine kinases of the Src family, and transmembrane proteins, are located in lipid rafts. Lipid raft localized proteins have been shown to be involved in intracellular membrane trafficking and signaling.

FOCUS™ Signal Proteins kit fractionates lipid raft localized proteins from other cellular proteins by employing non ionic detergents. Signal Protein Extraction Buffer is a proprietary formulation of non-ionic detergents designed to efficiently extract and remove soluble proteins, leaving lipid rafts containing signal proteins as a detergent insoluble fraction. The resulting rafts are then solubilized in FOCUS™ Protein Solubilization Buffer, a supplied 2D electrophoresis compatible buffer or a different buffer of choice.

FOCUS™ Membrane Protein kit is designed for >50 preps, where one prep is:

- 100mg mammalian tissue
- 50µl wet animal cell pellet
- 50µl wet yeast pellet
- 50µl wet bacteria pellet
- 250mg plant tissue

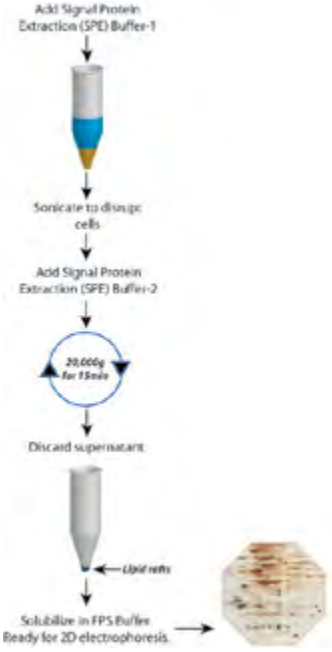

Figure 24: FOCUS™ Signal Protein scheme.

FEATURES

- Fractionate lipid raft localized proteins
- A 2D electrophoresis compatible buffer is provided

APPLICATIONS

- Isolate signal proteins that are localized to lipid rafts
- Study movement of activated proteins to and from lipid rafts
- Suitable for 2D gel electrophoresis, proteomics and cell signaling studies

CITED REFERENCES

Bodas, M. et al (2011) J. Immunol. 186: 602-613

Cat. No.	Description	Size
786-250	FOCUS™ Signal Proteins	50 preps

FOCUS™ PhosphoRich™

FOCUS™ PhosphoRich™ is a ready-to-use kit that enriches phosphorylated proteins and phosphopeptides from complex biological samples. The kit contains spin columns that have a phosphoprotein binding resin with a binding capacity of ~20mg phosphorylated ovalbumin per column. The resin columns supplied with the kit can be reused, if regenerated and stored properly.

Figure 25: Various concentrations of phosphoprotein were loaded onto the FOCUS™ PhosphoRich™ columns and were washed extensively. The protein was rapidly eluted and the eluted proteins were resolved by SDS-PAGE. The phosvitin was visualized with the Reversible Zinc Stain™.

FEATURES

- Uses a phosphorylated protein binding spin column
- Rapid binding and elution of phosphoproteins

APPLICATIONS

- Enrichment of phosphorylated proteins and peptides
- Suitable for wide range of downstream applications, including 1D & 2D electrophoresis, Western blotting and mass spectrometry
- Suitable for proteomics and cell signaling studies

Cat. No.	Description	Size
786-255	FOCUS™ PhosphoRich™	5 Preps

PhosphoQuant™

Estimation of phosphates in phosphoproteins

PhosphoQuant™ is specifically designed for quick and reliable determination of whether a purified protein is phosphorylated and the extent of phosphorylation. The assay is based on the alkaline hydrolysis of phosphates from seryl and threonyl residues in phosphoproteins and the subsequent quantification of the released phosphate with a Molybdate dye.

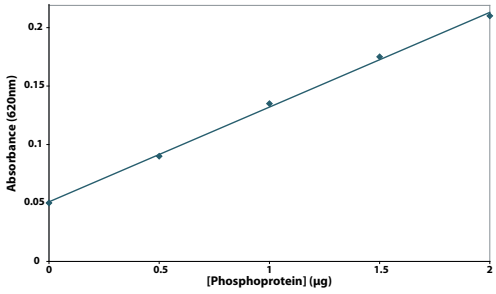

Figure 26: Standard calibration plot generated with PhosphoQuant™.

Cat. No.	Description	Size
786-256	PhosphoQuant™	400 assays

AlbuminOUT™

Samples that contain a large abundance of albumin, such as plasma and cerebrospinal fluid, tend to mask identification and discovery of other less abundant proteins in 2D gel electrophoresis and other studies. AlbuminOUT™ has been specifically developed for substantial removal of albumin from such samples.

The albumin removal method is based on binding of albumin with Cibachron™ Blue dye. AlbuminOUT™ has been optimized for removal of human albumin from samples. AlbuminOUT™ uses a rapid spin column method, where each column contains 0.2ml dye bound resins with capacity of >2mg human albumin per column. AlbuminOUT™ will remove >98% albumin from 5-50µl human plasma.

Spin column format allows removal of albumin within 10 minutes. High capacity blue-dye binding resin allows instantaneous binding and removal of albumin from human, pig, sheep, dog, rabbit, rat, and bovine samples. AlbuminOUT™ may also be used for removal of albumin from other species. Suitable for processing 25 or 50 samples.

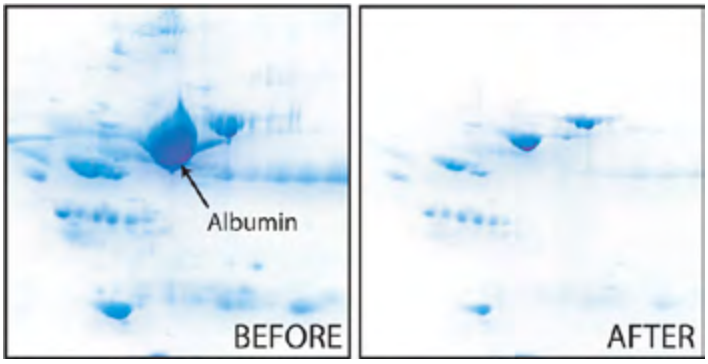

Figure 27: 2D analysis of whole human serum before (left) and after (right) treatment with AlbminOUT™.

FEATURES

- Removal of albumin from samples in less than 10 minutes
- Based on binding of albumin with Cibachron™ Blue dye
- Column capacity >2mg human albumin per column
- Removes >98% albumin from 5-50µl human plasma

APPLICATIONS

- Removal of albumin from biological samples such as plasma and cerebrospinal fluid

CITED REFERENCES

Sandilands, E.A. et al (2012) BMC Clin. Pharmacol. 12:3
De Palma, A. et al (2010) J. Chroma A. 1217:5328

Cat. No.	Description	Size
786-251	AlbuminOUT™	25 preps
786-252	AlbuminOUT™	50 preps

HOOK™ Cell Surface Protein Isolation Kit

HOOK™ Cell Surface Protein Isolation kit uses HOOK™ biotin labeling and purification technology in conjunction with our Mammalian Cell PE LB™ lysis buffer to conveniently label cell surface proteins and isolate them for further analysis.

Mammalian cells, adherent or non-adherent, are labeled with HOOK™ Sulfo-NHS-SS-Biotin, an amine reactive biotinylation reagent that is soluble in water, but impermeable to plasma membranes. HOOK™ Sulfo-NHS-SS-Biotin has a disulfide bond in the spacer arm that permits the cleavage of the biotin moiety from the protein, making its interaction with streptavidin purification column reversible.

Cells are lysed with Mammalian Cell PE LB™ and applied to a Streptavidin agarose column. Unlabeled intracellular proteins are washed away and the biotin labeled cell surface proteins are then released by reduction of the disulfide bond with DTT.

The kit is supplied with all the necessary reagents and buffers for convenience and improved reproducibility. The kit is compatible with a wide variety of mammalian cells and can be used to compare treated and untreated cells and differences between different cell lines. This kit is supplied with sufficient reagents for five experiments, with each experiment consisting of four 90-95% confluent T-75cm^2 flasks.

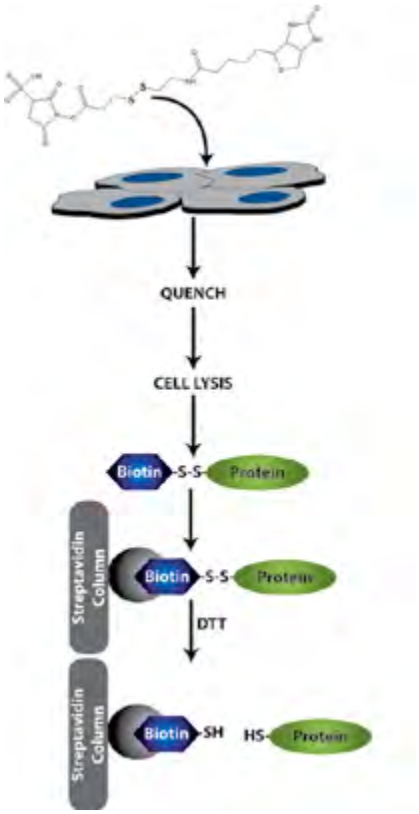

Figure 28: HOOK™ Cell Surface Protein Isolation scheme.

FEATURES

- Complete cell surface biotin labeling and isolation
- Suitable for a wide selection of mammalian cells

APPLICATIONS

- For cell surface trafficking & receptor:ligand interactions

CITED REFERENCES

Hartz, et al (2011) J. Euk. Microbio. 58:171
Bizet, A.A. et al (2011) BBA-Mol. Cell. Res. 1813:742

Cat. No.	Description	Size
786-316	HOOK™ Cell Surface Protein Isolation	5 Expts

DIALYSIS SYSTEMS

Dialysis is a popular technique used for the exchange of buffer medium across semi-permeable membranes. Dialysis devices are available in many configurations for research applications. We offer innovative dialysis devices and accessories for processing small samples.

Tube-O-DIALYZER™

Efficient dialysis with 100% sample recovery

Small sample dialysis has become a routine and popular technique in life science research. Today's major concern with dialysis devices is the loss of precious samples, due either to leaking or precipitation of samples during dialysis. A second concern is the efficiency and rate of dialysis. We manufacture a unique dialysis device that allows efficient dialysis and 100% sample recovery, even if your sample precipitates.

The unique tube format of Tube-O-DIALYZER™ allows for easy handling and manipulation. For sample recovery, just place the Tube-O-DIALYZER™ in a centrifuge and spin your sample to the bottom of the tube, ensuring 100% sample recovery, even if precipitation occurs.

The unique tube format also allows for easy sample loading, as simple as transferring your sample to a microcentrifuge tube. Tube-O-DIALYZER™ does not require the use of specialized loading devices or costly syringes and hazardous needles.

Tube-O-DIALYZER™ comes in two ideal sizes; the Micro unit allows efficient dialysis of 20-250µl samples and the Medi unit is optimized for 200µl-2.5ml samples. Both sizes are available with membranes with molecular weight cutoff (MWCO) of 1kDa, 4kDa, 8kDa, 15kDa and 50kDa. Tube-O-DIALYZER™ are available in packs of 20. Each Tube-O-DIALYZER™ is supplied with 6 floats and Tube-O-DIALYZER™ storage caps to allow storage of dialyzed samples. For added convenience, Tube-O-DIALYZER™ is also supplied as a mixed kit containing 10 Micro and 10 Medi Tube-O-DIALYZER™, along with the required floats and storage caps.

A graph representing the fast and highly efficient dialysis rate of the micro Tube-O-DIALYZER™ is shown. 100µl 5M NaCl was dialyzed against one liter deionized water. Samples were taken at specific times and the conductivity was measured. The graph demonstrates the fast efficiency of Tube-O-DIALYZER™, with 50% NaCl removed within 10 minutes.

APPLICATIONS

- Dialysis of small sample volumes
- Equilibrium dialysis for buffer exchange
- Concentration of samples
- Dialysis for single use applications, such as radioactive samples

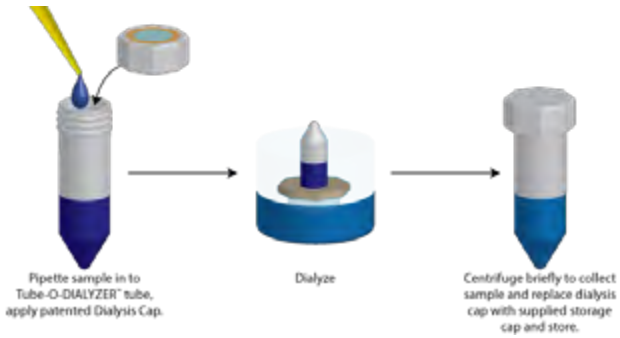

Figure 29: A summary of the Tube-O-DIALYZER™ system.

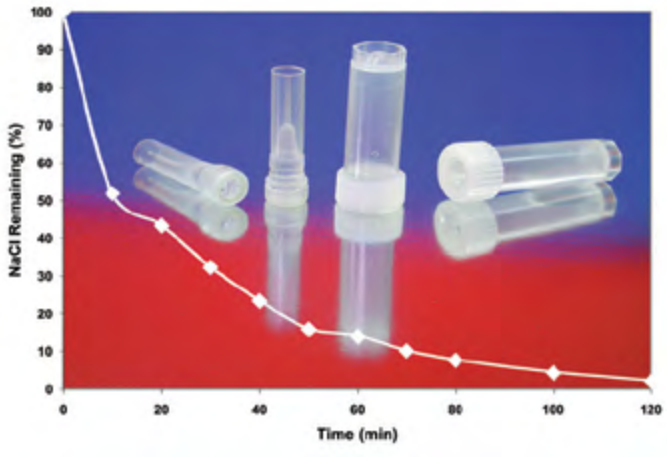

Figure 30: Tube-O-DIALYZER™ micro (8K MWCO) Dialysis Rate. 100µl 5M sodium chloride was dialyzed against 1 liter deionized water. 50% sodium chloride is removed in the first 10 minutes.

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Kubo, T. et al (2011) Langmuir. 27:9372
More citations available at www.GBiosciences. com

Cat. No.	Description	Size
786-610	Tube-O-DIALYZER™, Micro, 1k MWCO	20
786-610T	Tube-O-DIALYZER™, Micro, 1k MWCO	5
786-611	Tube-O-DIALYZER™, Micro, 4k MWCO	20
786-611T	Tube-O-DIALYZER™, Micro, 4k MWCO	5
786-612	Tube-O-DIALYZER™, Micro, 8k MWCO	20
786-612T	Tube-O-DIALYZER™, Micro, 8k MWCO	5
786-613	Tube-O-DIALYZER™, Micro, 15k MWCO	20
786-613T	Tube-O-DIALYZER™, Micro, 15k MWCO	5
786-614	Tube-O-DIALYZER™, Micro, 50k MWCO	20
786-614T	Tube-O-DIALYZER™, Micro, 50k MWCO	5
786-615	Tube-O-DIALYZER™, Medi, 1k MWCO	20
786-615T	Tube-O-DIALYZER™, Medi, 1k MWCO	5
786-616	Tube-O-DIALYZER™, Medi, 4k MWCO	20
786-616T	Tube-O-DIALYZER™, Medi, 4k MWCO	5
786-617	Tube-O-DIALYZER™, Medi, 8k MWCO	20
786-617T	Tube-O-DIALYZER™, Medi, 8k MWCO	5
786-618	Tube-O-DIALYZER™, Medi, 15k MWCO	20
786-618T	Tube-O-DIALYZER™, Medi, 15k MWCO	5
786-619	Tube-O-DIALYZER™, Medi, 50k MWCO	20
786-619T	Tube-O-DIALYZER™, Medi, 50k MWCO	5
786-620	Tube-O-DIALYZER™, Mixed, 1k MWCO	20
786-621	Tube-O-DIALYZER™, Mixed, 4k MWCO	20
786-622	Tube-O-DIALYZER™, Mixed, 8k MWCO	20
786-623	Tube-O-DIALYZER™, Mixed, 15k MWCO	20
786-624	Tube-O-DIALYZER™, Mixed, 50k MWCO	20

Tube-O-Reactor™

For protein cross-linking & modification reactions

Tube-O-Reactor™ is a system that allows all the key steps of cross-linking, coupling and modification of proteins and/or nucleic acids to be performed in a single tube. This minimizes the risk of sample loss, experimental time and hands-on phases.

Most of the above reactions involve three main steps:

1. Equilibration of reaction conditions for optimized reactions
2. Subsequent reaction with target agents (i.e. cross-linkers and labels)
3. Removal of unreacted agents and by-products

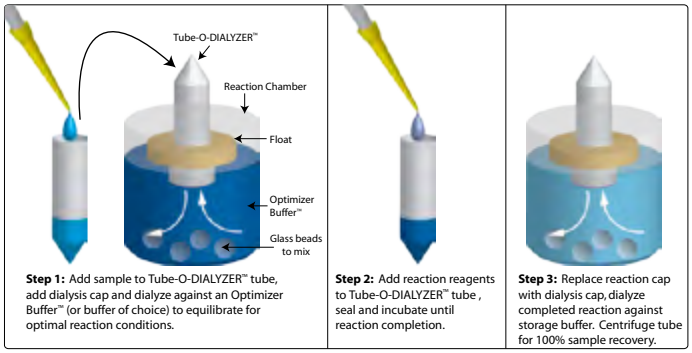

Figure 31: Tube-O-Reactor™ system.

The Tube-O-Reactor™ system is available in three MWCO sizes, 4kDa, 8kDa and 15kDa. Tube-O-Reactor™ is supplied as a Micro kit for sample sizes of 20-250µl and a Medi size for samples of 0.2-2.5ml.

Each Tube-O-Reactor™ is suitable for 5 reactions, depending on sample volumes, and is supplied with:

- 5 Medi or 5 Micro Tube-O-DIALYZER™
- 5 Floats for each size of Tube-O-DIALYZER™
- 5 Micro Dialysis Reaction Chambers
- Glass balls

Cat. No.	Description	Size
786-024-4k	Tube-O-Reactor™ (Micro), 4kDa MWCO	5 units
786-024-8k	Tube-O-Reactor™ (Micro), 8kDa MWCO	5 units
786-024-15k	Tube-O-Reactor™ (Micro), 15kDa MWCO	5 units
786-027-4k	Tube-O-Reactor™ (Medi), 4kDa MWCO	5 units
786-027-8k	Tube-O-Reactor™ (Medi), 8kDa MWCO	5 units
786-027-15k	Tube-O-Reactor™ (Medi), 15kDa MWCO	5 units

DIALYZER-Enhance™

For the dialysis of up to 12 samples at one time

Dialysis is the process of separating molecules in solution by the difference in their rates of diffusion through a semi permeable membrane, such as dialysis tubing or Tube-O-DIALYZER™ dialysis caps. Molecules small enough to pass through the dialysis membrane move across the membrane in the direction of decreasing concentration, until an equilibrium has been reached. In order to remove the highest amount of small molecules as possible, the dialysis must be performed against large volumes of dialysis buffers and/or require frequent changes of buffer to shift the equilibrium. In fact, the approximate maximal extent a small molecule can be removed by dialysis is estimated by: $(Vi/Vo)^{\#C}$, where Vi is the volume inside a dialysis bag; Vo is the volume of dialysis buffer and #C is the number of times the buffer is changed.

DIALYZER-Enhance™ is a proprietary product that when added to the dialysis buffer shifts the equilibrium resulting in the increased removal of a wide range of small molecules. The DIALYZER-Enhance™ consists of unreactive reagents that will not interfere or modify your reagents and will not cross the dialysis membrane, ensuring a pure, clean sample at the end of dialysis.

DIALYZER-Enhance™ is designed for use with our patented Tube-O-DIALYZER™ micro dialysis devices, dialysis tubing and bags for rapid and complete dialysis. 100X concentrated suspension suitable for 5 liters of dialysis buffer.

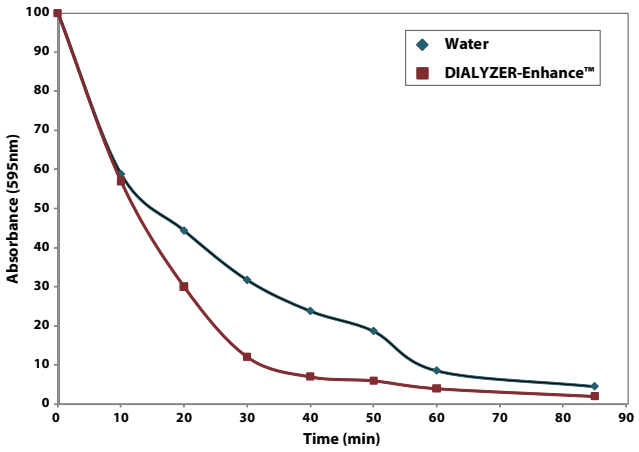

Figure 32: DIALYZER-Enhance™ reduces dialysis times. 0.5ml 5M NaCl was placed in a 8,000 MWCO Tube-O-DIALYZER™ dialyzed against 20ml water or 20ml water supplemented with DIALYZER-Enhance™.

FEATURES

- Unreactive dialysis enhancer
- Improve dialysis rates
- Increase removal of small molecules
- 100X suspension suitable for up to 5L dialysis buffer

APPLICATIONS

- For the enhancement of diaysis rates
- For the improved removal of small waste products
- Fully compatible with our Tube-O-DIALYZER™ range

Cat. No.	Description	Size
786-627	DIALYZER-Enhance™	50ml

TUBE-O-DIALYZER™ ACCESSORIES

Tube-O-Array™

For the dialysis of up to 12 samples at one time

This is a low cost system that allows for the rapid equilibration of samples in minimal buffer, requires minimal hands-on manipulation and can be used for 1-12 samples. Tube-O-Array™ consists of Tube-O-Array™ tray for supplied 12 Micro dialyzer cups. Simply add Tube-O-DIALYZER™ (supplied separately) and appropriate buffers.

APPLICATIONS

- Dialysis of multiple samples
- Ideal for equilibrium dialysis

Centrifuge Tube-Adapter

For centrifugation of Medi and Micro Tube-O-DIALYZER™ in a bench top centrifuge.

Tube-O-Tanks

Two dialysis tanks specifically designed for use with the Tube-O-DIALYZER™. Two sizes are available that are suitable for Micro and Medi size Tube-O-DIALYZER™.

Micro Dialysis Cups

For dialysis of small sample volumes, equilibrium dialysis, dialysis of single use preparations, and other dialysis applications. The Micro Dialysis Cup has dialysis buffer capacity of 2-15 ml.

Stirring Balls

Recommended for use with Micro Dialysis Cups for stirring dialysis buffer during dialysis. Supplied as 500 stirring balls.

Floats

Replacement Tube-O-DIALYZER™ floats are also available separately. Floats for Tube-O-DIALYZER™ Micro and Medi sizes are available. The floats for Micro are available in two sizes: 82021-312 is designed for dialysis in Tube-O-Tanks or a beaker and 82021-336 is designed for dialysis in the Micro Dialysis Cups.

Cat. No.	Description	Size
786-145A	Tube-O-Array™	1 kit
786-145	Tube-O-DIALYZER™ Centrifuge Tube Adapter	2
786-145D	Tube-O-Tanks (Small)	1
786-145E	Tube-O-Tanks (Large)	1
786-145C	Micro Dialysis Cups	12
786-145B	Stirring Balls	500
786-141F	Tube-O-DIALYZER™ Floats (Micro)	6
786-149	Tube-O-DIALYZER™ Floats (Micro for Dialysis Cups)	12
786-142F	Tube-O-DIALYZER™ Floats (Medi)	6

DESALTING & BUFFER EXCHANGE

Spin-OUT™

For desalting and buffer exchange

The SpinOUT™ GT-100, GT-600 and GT-1200 columns are versatile, spin-format columns for the desalting and buffer exchange of protein and nucleic acid solutions ranging from 5µl through to 4ml sample volumes. The SpinOUT™ columns are available in three MWCO sizes for >700, >6,000 or >30,000 Dalton peptides or proteins and are suitable for samples containing as little as 20µg protein/ml.

Easy to use; simply apply the protein sample and centrifuge to recover proteins and nucleic acids with the column retaining more than 95% of the salts and small molecules (>100Da for GT-100, <1,000Da for GT-600 and <1,500Da for GT-1200).

Spin-OUT™ GT-100 for purification of peptides & proteins >700Da.

Spin-OUT™ GT-600 for the purification of proteins >6kDa and nucleic acids larger than 10bp.

Spin-OUT™ GT-1200 is for the purification of proteins >30kDa and removal of molecules >1,500Da. The columns are ideal for separating proteins from peptides.

FEATURES

- 5 sizes available for sample volumes of 5µl to 4ml
- Spin format for rapid purification

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Cat. No.	Description	Size	Resin Bed (ml)	Sample Load (ml)
786-865	SpinOUT™ GT-100, 0.1ml	25 columns	0.1	0.005-0.02
786-866	SpinOUT™ GT-100, 1ml	10 columns	1	0.05-0.1
786-867	SpinOUT™ GT-100, 3ml	10 columns	3	0.1-0.5
786-868	SpinOUT™ GT-100, 5ml	5 columns	5	0.5-2
786-869	SpinOUT™ GT-100, 10ml	5 columns	10	0.5-4
786-703	SpinOUT™ GT-1200, 0.1ml	25 columns	0.1	0.005-0.02
786-170	SpinOUT™ GT-600, 1ml	10 columns	1	0.05-0.1
786-171	SpinOUT™ GT-600, 3ml	10 columns	3	0.1-0.5
786-704	SpinOUT™ GT-600, 5ml	5 columns	5	0.5-2
786-705	SpinOUT™ GT-600, 10ml	5 columns	10	0.5-4
786-706	SpinOUT™ GT-1200, 0.1ml	25 columns	0.1	0.005-0.02
786-172	SpinOUT™ GT-1200, 1ml	10 columns	1	0.05-0.1
786-173	SpinOUT™ GT-1200, 3ml	10 columns	3	0.1-0.5
786-707	SpinOUT™ GT-1200, 5ml	5 columns	5	0.5-2
786-708	SpinOUT™ GT-1200, 10ml	5 columns	10	0.5-4

SpinOUT™ for PCR

SpinOUT™ PCR is for the cleaning of PCR products. PCR-20 removes contaminating products from PCR products, including <20bp primers, dNTPs and salts. PCR-32 removes PCR products from <32bp primers, dNTPs and salts. For more information see the DNA Clean Up & Concentration section.

Cat. No.	Description	Size
786-174	SpinOUT™ PCR-20	10 columns
786-175	SpinOUT™ PCR-32	10 columns

DETERGENT REMOVAL

G-Biosciences offers a range of detergent removal systems that use either a rapid column based system or a precipitation system.

Our products are designed to remove a wide variety of detergents, including SDS, Tween® 20, Triton® X-100, Triton® X-114, Nonidet® P-40, CTAB, CHAPS, deoxycholate and Lubrol®.

DetergentOUT™ GBS10

Detergents are essential for protein solubility during protein extraction and sample preparation, especially when working with hydrophobic proteins. The presence of high concentrations of detergents in protein samples can impair ELISA, IEF, protease digestion of proteins and suppress peptide ionization when analyzed by mass spectrometry.

The resin removes free, unbound anionic, nonionic or zwitterionic detergents (e.g. SDS, Triton® X-100 or CHAPS) from aqueous protein and peptide samples with minimal sample loss for downstream analysis by mass spectrometry and other techniques.

The DetergentOUT™ GBS10 columns were shown in independent studies to be fully compatible with DI-QTOF and LC-MS/MS (see references). The use of the DetergentOUT™ GBS10 columns significantly increased the number of peptide spectra detected. In addition, the DetergentOUT™ GBS10 columns have a high binding capacity for detergents, i.e. 6mg SDS and 14mg Triton® X-100 by every ml settled resin.

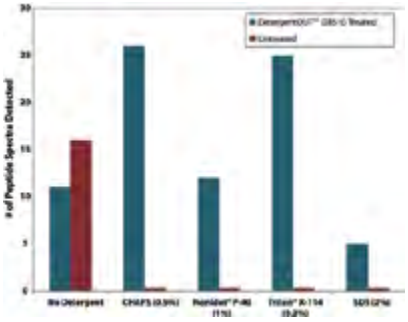

Figure 33: DetergentOUT™ GBS10 removes detergent & allows detection of peptide fragments by mass spectrometry. 500µg phosphorylase B was digested in solution & then the indicated amount of detergent was added. Samples were treated with DetergentOUT™ GBS10. Number of peptide spectra were determined as per the protocol of Alvarez, S. et al.

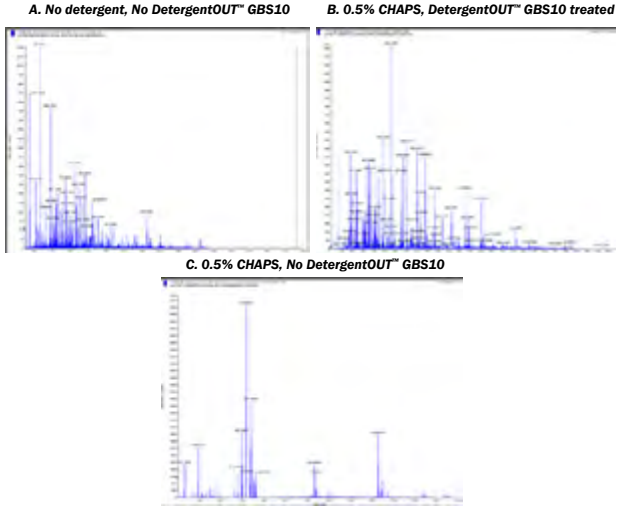

Figure 34: DetergentOUT™ GBS10 enhances mass spectrometry spectra. 5µg/µl protein mixture (BSA, cyctochrome C and phosphorylase B) in water (Panel A) was supplemented with 0.5% CHAPS (Panel B and C). The CHAPS containing sample was treated with DetergentOUT™ GBS10 and compared to an untreated sample (Panel C). Spectra were generated per Alvarez et al.

Detergent	% Removed	BSA	Phosphorylase B	Cytochrome C	E.coli Lysate
Triton X-100, 2%	>99	>90	>91	>92	>93
Triton X-114, 2%	>96	>99	>98	>97	>91
Nonidet P-40, 1%	>96	>93	>95	>91	>91
Brij 35, 1%	>99	>98	>99	>97	>91
SDS, 2.5%	>99	>96	>97	>92	>90
Sodium deoxycholate, 5%	>99	>99	>99	>98	>95
CHAPS, 3%	>99	>92	>95	>92	>91
Octyl glucoside, 5%	>99	>93	>95	>96	>91
Lauryl maltoside, 1%	>97	>99	>99	>99	>91

Table 2: A comparison of the detergent removal rates and percentage protein recovery with DetergentOUT™ GBS10.

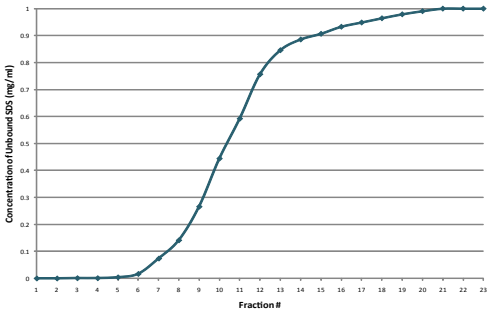

Figure 35: DetergentOUT™ GBS10 retains ≤6mg SDS per ml settled resin. SDS solution was continuously applied to DetergentOUT™ GBS10 column. The graph depicts the amount of SDS detected in the flow-through. SDS was not detected until fraction 7, so after 12mg SDS had been retained by the 2ml of DetergentOUT™ GB-S10 resin, resulting in a 6mg/ml settled resin binding capacity.

CITED REFERENCES

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Baizman, E. et al (2000) Microbiology 146: 3129
Hou, S. et al (2013) Methods. 61:2697
Hashii, N. et al (2005) Proteomics. 5:4665

Cat. No.	Description	Sample Size (µl)	Resin (µl)	Size
786-154	DetergentOUT™ GBS10-125	10-30	125	10 columns
786-155	DetergentOUT™ GBS10-800	30-200	800	10 columns
786-156	DetergentOUT™ GBS10-3000	200-750	3,000	10 columns
786-157	DetergentOUT™ GBS10-5000	500-1,250	5,000	10 columns
786-159	DetergentOUT™ GBS10 Resin	-	-	10ml resin

DetergentOUT™ Tween®

Removal of Tween® (polysorbate) detergents

A spin column format detergent removal resin for polysorbate or Tween® detergents or surfactants. DetergentOUT™ Tween® specifically removes polysorbate detergents without significant loss of proteins, dilution of the protein solution, or change to the buffer composition of the protein solution.

For other detergents, we highly recommend our DetergentOUT™ GBS10 columns and resin. The DetergentOUT™ GBS10 shows greater efficiency of detergent removal and protein recovery for other detergents, including SDS, CHAPS, Triton®, Nonidet® and Brij®

Cat. No.	Description	Size
786-214	Detergent*OUT*™ Tween®, Micro	10 columns
786-215	Detergent*OUT*™ Tween®, Medi	10 columns

OrgoSol DetergentOUT™

Suitable for hydrophobic proteins, removes detergents and concentrates protein solutions

OrgoSol DetergentOUT™ is suitable for removal of detergents from protein solutions, including hydrophobic protein solutions and is compatible with all detergent types. Its performance is not dependent on the concentration of detergents in the solution, is highly flexible and can be used to process small and large sample volumes.

OrgoSol DetergentOUT™ first concentrates the protein solution through precipitation and then the detergent is extracted and removed with the supplied OrgoSol™ buffer. The proprietary precipitation agent ensures >99% protein recovery, however precipitation may result in some loss of a protein's biological activity.

Two sizes are offered: Micro Kit for processing up to a total of 10ml protein solution and Medi Kit for processing up to a total of 30ml protein solution, either in a single or multiple experiments.

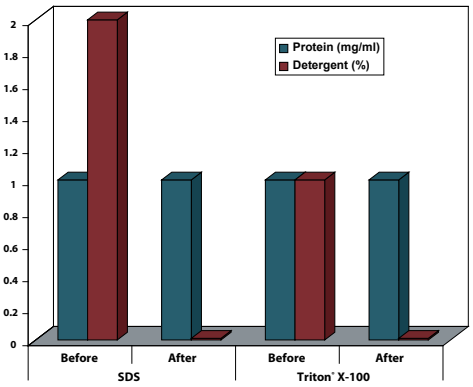

Figure 36: Removal of Detergent. Hydrophobic nuclear fraction proteins (1mg/ml) in 2% SDS and 1% Triton® X-100 before and after OrgoSol DetergentOUT™ treatment.

CITED REFERENCES

Cortes, D.F. et al (2012) Electrophoresis. 33:3712
Orr, S.J. et al (012) Molecular Sys Biol. DOI: 10.1038/msb.2012.5
Troese, M.J. et al (2011) Infect Immunol. 79:4696

Cat. No.	Description	Size
786-127	OrgoSol Detergent*OUT*™, Micro	For 10ml
786-128	OrgoSol Detergent*OUT*™, Medi	For 30ml

ENDOTOXIN REMOVAL

EndotoxinOUT™

Rapid removal of endotoxins & pyrogens

For the rapid removal of endotoxins/pyrogens (LPS, lipopolysaccharides) from samples.

EndotoxinOUT™ consists of 6% cross-linked agarose covalently linked to polymyxin B to bind and remove harmful pyrogens from a solution. Polymyxin B is a family, polymyxin B1 and B2, of antibiotics that bind to the negatively charged site of the lipid A portion of bacterial lipopolysaccharide layer neutralizing the endotoxic activity.

The covalent coupled agarose and polymyxin B is a stable matrix that resists leaching. An ideal product for the clean up of buffers, cell culture media, protein solutions, nucleic acid (DNA) samples and pharmacological components.

FEATURES

- Polymyxin B Sulfate immobilized on 6% cross-linked agarose
- Capacity: ≥9995 endotoxin units (EU) removed by 1ml resin from 5ml test containing 10,000EU
- ≥99.95% removal
- Reusable at least 10 times

APPLICATIONS

- Clean up of buffers, cell culture media, protein solutions and pharmacological components
- Removal of endotoxins from nucleic acid (DNA) samples

Cat. No.	Description	Size
786-367	EndotoxinOUT™	10ml resin
786-368	EndotoxinOUT™	1L resin
786-369	EndotoxinOUT™	5 x 1ml columns

UPPA-PROTEIN-Concentrate™

Rapid precipitation & concentration

UPPA PROTEIN-Concentrate™ uses a proprietary reagent, UPPA™ (Universal Protein Precipitation Agent), to quantitatively concentrate dilute protein samples as low as 1ng/ml. Concentration is not affected by the presence of common laboratory agents, including detergents and chaotropes. After precipitation, the sample is washed to remove salts and other interfering agents; complete recovery of sample is produced. Protein samples have conductivity of ~50µS and ~100% recovery.

UPPA PROTEIN-Concentrate™ kit is available as a Micro kit for concentrating up to 10ml of dilute protein solutions; and a Medi Kit for concentrating up to 30ml of dilute protein solutions, either as a single or multiple procedures.

FEATURES

- Concentrate as dilute as 1ng/ml
- Removes non-protein agents
- Low conductivity, ~ 50µS
- 100% sample recovery

APPLICATIONS

- For concentrating proteins for running gels, raising antibodies, protein purification, protein assays, and other applications
- This kit contains an acidic component and may not be suitable for those proteins which may lose some of their biological activities when precipitated

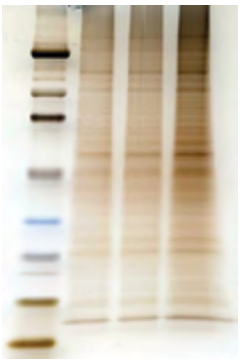

Figure 37: Concentration of dilute mouse liver lysate. Lane 1: Protein Marker; Lane 2: 20µl dilute protein (0.05µg/µl). Lane 3: 20µl original sample treated with UPPA-PROTEIN-Concentrate™ and resuspended in 20µl. Lane 4: 40µl original sample treated with UPPA-PROTEIN-Concentrate™ and resuspended in 20µl. Comparing lanes 2 and 3 shows that there is 100% protein recovery and lane 4 shows the actual concentration of a sample.

CITED REFERENCES

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Singleton, D. et al (2004) Microbiol 150:285
Chen, C. et al (2003) Mol. Microbiol. 49:1657
Morisawa, G. et al (2000) Plant Cell 12:1903

Cat. No.	Description	Size
786-120	UPPA-PROTEIN-Concentrate™ (Micro)	For 10ml sample
786-121	UPPA-PROTEIN-Concentrate™ (Medi)	For 30ml sample

UPPA-I & II Pack

UPPA™ (Universal Protein Precipitation Agent) is offered separately for the concentration of dilute (>1ng/ml) protein solutions. Concentration of proteins with UPPA™ is unaffected by chaotropes, detergents or common laboratory reagents.

Cat. No.	Description	Size
786-122	UPPA™-I & II Pack	For >80ml

OrgoSol-PROTEIN-Concentrate™

Preserve biological activity during concentration

The OrgoSol-PROTEIN-Concentration™ kit precipitates protein with a proprietary solvent buffer, OrgoSol™. The OrgoSol™ buffer has been specifically developed for efficient precipitation of protein solutions with minimal disruption to the protein structure and therefore maintains the biological activity of most proteins.

The kit has been extensively tested for the concentration of a wide selection of enzymatic proteins without the loss of their biological activity and for ~100% protein recovery. The kit is designed to precipitate up to 5ml protein solution.

The method involves mixing a protein solution with the OrgoSol™ Buffer followed by incubation, which results in quantitative precipitation of the protein. The precipitated protein is suspended in a smaller volume of an appropriate buffer and the concentrated protein solution is ready for use.

FEATURES

- Precipitates enzyme proteins without loss of activity
- Uses a proprietary organic solvent buffer
- Recovery ~100%

CITED REFERENCES

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Shah, R.N. et al (2012) Adv. Hematol. http://dx.doi.org/10.1155/2012/596925
Olbrot, M. et al (2002) PNAS 99:6737

Cat. No.	Description	Size
786-125	OrgoSol-PROTEIN-Concentrate™	For 5ml protein

Column-PROTEIN-Concentrate™

For larger volumes of dilute protein solutions

The Column-PROTEIN-Concentrate™ kit has been specifically developed for concentration of those proteins that cannot be concentrated by precipitation. The kit is based on a proprietary Protein Binding Resin that binds and immobilizes any protein in a low salt buffer between pH 2-12. The binding capacity is ~0.5mg protein/ml Protein Binding Resin.

The immobilized protein is spin-eluted in a small volume of specifically formulated elution buffer, giving several fold effective concentration. The method is gentle and protects protein from denaturation during the concentration process.

Suitable for concentration of a total of 4mg protein in either single or multiple procedures. request further information for concentration of >5mg protein.

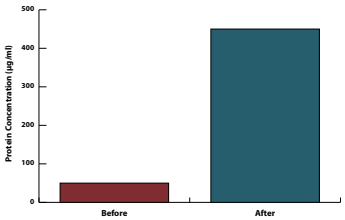

Figure 38: Column concentration of dilute proteins. 1ml dilute protein (50µg/ml) was concentrated with Column-PROTEIN-Concentrate™ to a final volume of 110µl with a concentration of 450µg/ml.

FEATURES

- Spin column format for concentration of proteins
- Reusable protein binding resin (capacity ~0.5mg protein/ml)
- Maintains and protects biological activity of proteins
- Recovery ~ 100%

CITED REFERENCES

Taggart, C et al (2005) J. Exp. Med. 202:1659

Cat. No.	Description	Size
786-126	Column-PROTEIN-Concentrate™	For 4mg protein

Tube-O-CONCENTRATOR™

Rapid concentration of proteins >1kDa without protein precipitation

Tube-O-CONCENTRATOR™ is a versatile concentration device that utilizes a novel, water absorbing, liquid polymer and our patented Tube-O-DIALYZER™ for the rapid concentration of dilute protein solutions with zero protein loss. The unique tube design of Tube-O-DIALYZER™ ensures that 100% sample is recovered; simple place the entire device in a bench top centrifuge and spin for a few seconds.

The Tube-O-CONCENTRATOR™ solution is a liquid polymer that rapidly absorbs water through the dialysis membrane in the Tube-O-DIALYZER™ cap, which retains all molecules with a molecular weight >1kDa.

Tube-O-CONCENTRATOR™ is available in two sizes for concentrating sample volumes of up to 250µl (Micro) or 2.5ml (Medi).

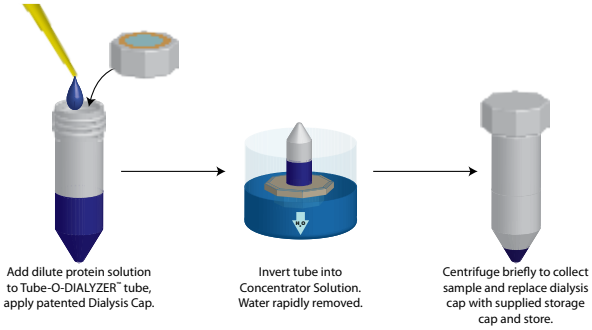

Figure 39: Tube-O-CONCENTRATOR™ scheme.

FEATURES

- Rapid concentration of >1kDa proteins without protein precipitation
- Combination of a unique tube format dialysis device and a water absorbing liquid polymer
- Suitable for up to 250µl or 2.5ml protein solutions
- Zero protein loss, even if protein precipitation occurs

CITED REFERENCES

Ildefonso, C. J. et al (2012) J. Biol. Chem. 287:32697

Cat. No.	Description	Size
786-625	Tube-O-CONCENTRATOR™ for 20-250µl	5 concentrations
786-626	Tube-O-CONCENTRATOR™ for 0.2-2.5ml	5 concentrations

Concentrator Solution

Concentrate proteins by dialysis

Concentrator Solution is a novel liquid polymer for the rapid concentration of dilute protein solutions with zero loss, using dialysis. Simply transfer your dilute protein solution to a dialysis bag or dialysis device, such as our patented Tube-O-DIALYZER™ and dialyze against the concentrator solution. The water will be rapidly removed through the dialysis membrane, which also retains your protein of interest and prevents the high molecular weight liquid polymer entering your solution. Once the desired volume of your solution is achieved, quickly rinse the excess concentrator solution from the dialysis bag/ membrane and recover your sample.

FEATURES

- Fast, efficient and clean protein concentration
- Minimal hands-on activity
- No protein loss
- Can be supplemented with buffers, salts and other chemicals

CITED REFERENCES

Ildefonso, C. J. et al (2012) J. Biol. Chem. 287:32697

Cat. No.	Description	Size
786-143	Concentrator Solution	125ml

Concentrator Powder

Concentrator Powder is a high molecular weight polymer which will not migrate across the dialysis membrane.

Simply transfer your dilute protein solution to a dialysis bag or dialysis device, such as our patented Tube-O-DIALYZER™ and then cover the membrane with Concentrator Powder. Concentrator Powder rapidly absorbs water from the sample and reduces the sample volume.

Cat. No.	Description	Size
786-144	Concentrator Powder	150g

G-Biosciences offers two specialized electrophoresis clean up products that remove electrophoretic interfering agents prior to 1D or 2D gel electrophoresis.

Perfect-FOCUS™

Streak free 2D gels & improved spot resolution

Designed to clean and concentrate protein samples that give poor protein spot resolution during 2D electrophoresis. Protein samples containing interfering agents, including ionic detergents, metal ions, substrates, substrate analogs, inhibitors, and other charged molecules, routinely result in smeared or low resolution gels. Protein solutions are treated with *Perfect*-FOCUS™ that quantitatively precipitates the proteins, which are subsequently collected by centrifugation. The non-protein interfering agents are washed away and the protein pellet is ready to be solubilized in an appropriate sample loading buffer.

The collected protein has conductivity <50µS and is substantially free from non-protein agents, which improves spot resolution and greatly reduces spot streaking. Compatible with mass spectrometry analysis.

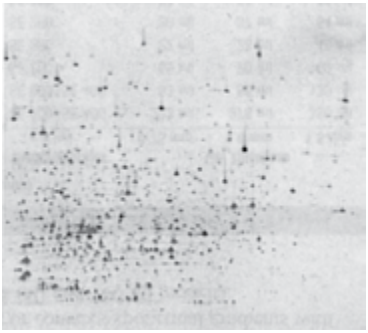

Figure 40: Treatment of protein samples with Perfect-FOCUS™.

FEATURES

- Removes interfering agents from protein solutions
- Conductivity lower than 50µS after treatment
- Samples free from non-protein agents
- Kit suitable for 50 x 1-100µl protein solutions

APPLICATIONS

- Suitable for concentrating and cleaning protein samples for isoelectric focusing (IEF) and 2D-gel electrophoresis

CITED REFERENCES

Kerns, P.W. et al (2014) Pathogens and Disease. DOI: 10.1111/2049-632X.12142
Kokjohn, T.A. et al (2013) J. Neurotraum. 30:981
Solazzo, C. et al (2013) Int. Biodeter. Biodegrad. 80:48
Fekkar, A. et al (2012) J. Infect Dis. 205:1163
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Wang, H. et al (2012) Method Mol. Biol. 876:83
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Devillard, E. et al (2004) J Bacteriol 186:136
Rincon, M. et al (2004) J Bacteriol 186:2576

Cat. No.	Description	Size
786-124	*Perfect*-FOCUS™	50 preps
786-124T	*Perfect*-FOCUS™	6 preps

PAGE-*Perfect*™

Improved protein resolution & publication quality gels

Many lysis buffers and reagents used in sample preparation are incompatible with routinely used electrophoretic analysis. The presence of contaminants, or interfering agents, such as salts, acids, bases and detergents, result in band distortion and poor protein resolution. As a result, SDS-PAGE gels are hard to analyze and lack reproducibility.

PAGE-*Perfect*™ is a simple, two-step method for concentrating, cleaning and preparing protein solutions for running publication quality gels. Treat (1-100µl) protein solution with Universal Protein Precipitation Agent (UPPA™), which results in quantitative precipitation of the protein solution. Protein precipitation is not affected by the presence of detergents, chaotropes, or other common laboratory agents. The protein precipitate is collected by centrifugation and washed to remove any interfering agents such as detergents, salts, lipids, and other laboratory agents. Suspend the precipitate in the sample-loading buffer for loading on the gel for electrophoresis.

The figure below demonstrates the effect of PAGE-*Perfect*™ on the clean-up of 10µg mouse liver lysate that contain the indicated contaminants.

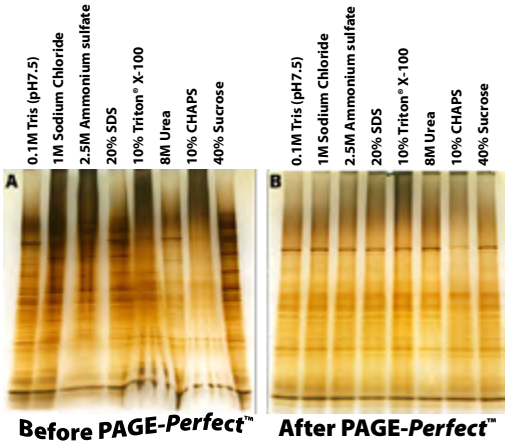

Figure 41: Analysis of mouse liver lysate before and after treatment with PAGE-Perfect™. A. 10µg mouse liver lysates, in the presence of various interfering agents, were loaded onto 4-20% SDS polyacrylamide gel. B. 10µg mouse liver lysates, in the presence of various interfering agents, were treated with PAGE-Perfect™ and then loaded onto 4-20% SDS polyacrylamide gel. Gels were stained with FASTsilver™ protein stain.

FEATURES

- Removes electrophoresis interfering agents, including:
  - Detergents • Salts • Chaotropes • Reducing agents • Sugars
- Concentrates and cleans dilute (>1ng/ml) protein samples
- Increase gel quality and reproducibility
- Protein recovery >99%
- Process 50 x 1-100µl protein samples

APPLICATIONS

- Suitable for cleaning and concentrating protein solutions for electrophoresis and other applications

CITED REFERENCES

Devillard, E. et al (2004) J Bacteriol 186:136
Rincon, M. et al (2004) J Bacteriol 186:2576
Kovacina, K. et al (2003) JBC 278:10189
Yi, F. et al (2003) Cancer Res. 63:2923
Grimaldi, M. et al (2003) J Neurosci 23:4737
Wu, X. et al (2002) JBC 277:13597

Cat. No.	Description	Size
786-123	PAGE-*Perfect*™	50 preps
786-123T	PAGE-*Perfect*™	5 preps