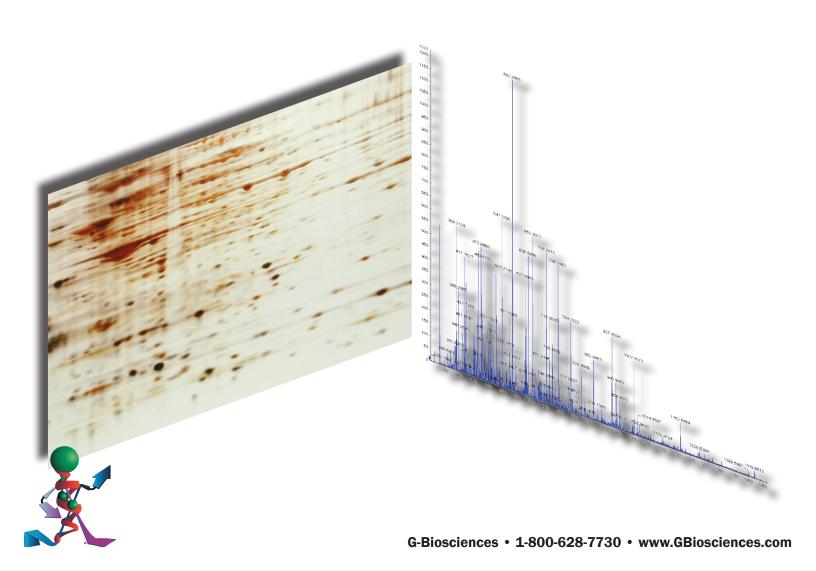
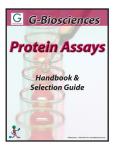


G G-Biosciences

Mass Spectrometry Sample Prep **Handbook & Selection Guide**





G G-Biosciences Protease &

Phosphatase

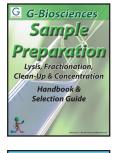
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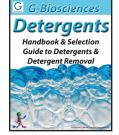
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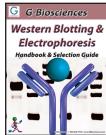
Handbook & Selection Guide

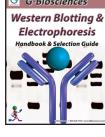
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- SAM Methyltransferase Assays
- **Protease Assays**
- **Phosphatase Assays**
- **Peroxide Assay**
- **Individual Protease Inhibitors**
- **Proteases for Mass Spec.**

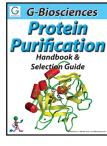
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- **Protein Fractionation Kits**
- Dialysis (Micro) System
- **Electrophoresis Clean-Up**
- **Concentration Systems**
- Contamination Removal

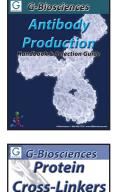


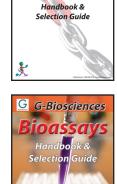






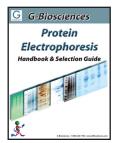






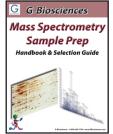


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- **Proteomic Grade Detergents**
- **Research Grade Detergents**
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- **Detergent Removal Systems**

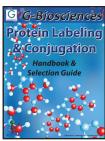


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- **Electrophoresis Buffers**
- Reducing & Alkylating Reagents
- **Protein Gel Stains**

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- **Transfer Buffers & Membranes**
- Membrane Stains
- **Blocking Buffers**
- **Secondary Antibodies**
- **Detection Reagents**
- Reprobing Reagents



- **Protein Sample Preparation**
- **Protein Clean-Up Systems**
- **Electrophoresis Reagents**
- **Mass Spec Grade Protease**
- **InGel Digestion Kits**
- **Peptide Generation Reagents**
- **Affinity Resins**
- **6X His Protein Purification Kits**
- **GST Protein Purification Kits**
- **Antibody Purification**
- **Activated Resins**
- **Buffers & Reagents**



- **Biotin Labeling**
- **Cell Surface Protein Labeling**
- **Agarose Coupling Kits**
- Fluorescent Dye Labeling Kits
- **Enzyme Labeling Systems**
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- **Peptide Coupling Systems**
- **Antibody Purification Resins**
- **Antibody Fragmentation Kits**



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Introduction

Mass spectrometry is an important method for the characterization and sequencing of proteins. The two primary methods for ionization of whole proteins are electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI).

Two approaches are used for characterizing proteins. In the first, intact proteins are ionized by either of the two techniques described above, and then introduced to a mass analyzer. This approach is referred to as "top-down" strategy of protein analysis.

In the second, proteins are enzymatically digested into smaller peptides using proteases such as trypsin, either in solution or in gel after electrophoretic separation. Other proteolytic agents are also used. The collection of peptide products are then introduced to the mass analyzer. When the characteristic pattern of peptides is used for the identification of the protein. These procedures of protein analysis are also referred to as the "bottom-up" approach.

There are several other forms of mass spectrometry ionization and detection available to researchers and this, combined with the vastness of proteomes makes sample preparation difficult and confusing. There is no single method suitable for the preparation of a protein for identification by mass spectrometry.

The protocols and products used in sample preparation vary greatly and are dependent on the starting sample, the experimental design and the mass spectrometry method used. Important factors to consider when designing your mass spectrometry experiments include, but are not limited to, protein source, type of protein, physical properties, abundance and cellular location.

A major issue to consider, which greatly improves the success of experiments, is the simplification of the proteome used in the analysis. The simpler the sample to be ionized the greater then chance of protein identification.

To aid researchers, G-Biosciences has developed products that are suitable for 2D electrophoresis and mass spectrometry and ensure simpler samples with minimal interfering agents.

Tissues & Cells **Cell Lysis & Protein Extraction**

Cell Lysis Buffers (Mild & Chaotropic) **Detergents Protease & Phosphatase Inhibitors**

Bodily Fluids High Abundant Protein Removal

AlbuminOUT™ Immobilized Protein A & Protein G

Fractionation & Enrichment

FOCUS™ Soluble & Insoluble Kit FOCUS™ Cytoplasmic & Nuclear Proteins FOCUS™ Membrane Proteins FOCUS™ Signal Protein FOCUS™ SubCell Kit FOCUS™ Mitochondria Kit FOCUS™ Global Fractionation FOCUS™ Glycoprotein FOCUS™ PhosphoRich[™] HOOK™ Cell Surface Protein Isolation Fraction-FOCUS

Clean-up & Concentration

Detergent Removal DetergentOUT™ GBS10 OrgoSol DetergentOUT™ DetergentOUT™ Tween®

Sample Clean-up Tube-O-DIALYZER™ SpinOUT™ Desalting Columns

OrgoSol-PROTEIN-Concentrate™ Column-PROTEIN-Concentrate™ Tube-O-CONCENTRATOR™

Concentration Systems Electrophoresis Clean-up Perfect-FOCUS™

Electrophoresis Separation

1D & 2D Electrophoresis Reagents

Mass Spectrometry Compatible Stains

LabSafe GEL Blue™ FOCUS™ FASTsilver™ Reversible Zinc Stain™

Peptide Generation

Trypsin, Mass Spectrometry Grade lmmobilized Trypsin InGel™ Silver InGel™ Blue

MILD EXTRACTION BUFFERS

PE LB™ Systems

Lysis systems for biologically active proteins

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.

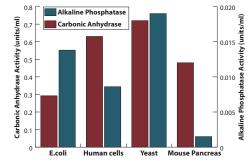


Figure 1: PE LB™ System maintains the biological activity of proteins. Extraction of carbonic anhydrase or alkaline phosphatase from E.coli, human cells, yeast and mouse pancreas with Bacterial, Mammalian Cell, Yeast and Tissue PE LB™ respectively. The resulting lysates retain their biological activity.

The PE LB™ systems offer a wide selection of buffers for lysis and extraction of proteins from bacteria, yeast, animal cells and tissues. 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.

The PE LB™ systems are compatible with most downstream applications including enzyme assays, ELISA, chromatographic applications, gel electrophoresis, Western blotting and protein folding procedures. An application note describing the use of the PE LB™ for the extraction of biologically active enzymes from various samples is available at GBiosciences.com.

CITED REFERENCES

- Barry, K.C. et al (2017) Global analysis of gene expression reveals mRNA superinduction is required for the inducible immune response to a bacterial pathogen. eLIFE. DOI: http://dx.doi. org/10.7554/eLife.22707
- Lee, C. M. et al (2016) Benzyl isothiocyanate inhibits inflammasome activation in E. coli LPSstimulated BV2 cells. Int. J. Mol. Med.DOI: 10.3892/ijmm.2016.2667

More cited references found at www.gbiosciences.com

Cat. No.	Description	Size
<u>786-176</u>	Bacterial PE LB [™] Kit including PE LB [™] Lysozyme	100 preps
<u>786-187</u>	Bacterial PE $LB^{\underline{\tiny{M}}}$ Kit including PE $LB^{\underline{\tiny{M}}}$ Lysozyme	250 preps
<u>786-188</u>	Bacterial PE $LB^{\underline{\tiny{M}}}$ Kit including PE $LB^{\underline{\tiny{M}}}$ Lysozyme	500 preps
<u>786-177</u>	Bacterial PE LB [™] buffer only	500ml
<u>786-185</u>	Bacterial PE LB [™] buffer only	100ml
<u>786-186</u>	Bacterial PE LB™ buffer only	250ml
<u>786-189</u>	Bacterial PE LB™ (2X) buffer only	250ml
<u>786-191</u>	Bacterial PE LB™ in Phosphate Buffer	500ml
<u>786-178</u>	Yeast PE LB™ Kit including Zymolyase®	100 preps
<u>786-179</u>	Yeast PE LB™, buffer only	500ml
<u>786-180</u>	Mammalian Cell PE LB™_	500ml
<u>786-181</u>	<u>Tissue PE LB</u> [™]	500ml
<u>786-411</u>	Insect PE LB™_	250ml

DENATURING CHAOTROPIC BUFFERS

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. Cvanate 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-touse 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. "Improved Protein **Extraction for 2D Electrophoresis by Sequential Solubilization** with FOCUS™ Chaotropic Extraction Buffers" is available at GBiosciences.com.

CITED REFERENCES

Walliwalagedara, C. et al (2010) Open Proteomics. 3:20

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

Cat. No.	Description	Major Components	Size
<u>786-220</u>	FOCUS [™] Extraction Buffer I	Urea & Nonidet® P-40	50ml
<u>786-221</u>	FOCUS [™] Extraction Buffer II	Urea & CHAPS	50ml
<u>786-222</u>	FOCUS [™] Extraction Buffer III	Urea, thiourea, CHAPS & ASB-16	50ml
<u>786-223</u>	FOCUS [™] Extraction Buffer IV	Urea, thiourea, CHAPS & SB 3-10	50ml
<u>786-219</u>	FOCUS [™] Extraction Buffer V	Urea, thiourea & CHAPS	50ml
<u>786-233</u>	FOCUS [™] Extraction Buffer VI	Urea, thiourea, CHAPS & NDSB 201	50ml
<u>786-234</u>	FOCUS [™] Extraction Buffers I-VI	1 vial of each buffer	10ml

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

Cell Lysis & Protein Extraction

RAB Buffer (Reassembly Buffer)

A high salt RAB (Reassembly) buffer for the lysis of mammalian cells, including CHO1,2, COS3, NT2N4,5 and HEK29310; C. elegans6,10 and brain tissue7-9.

FEATURES

 Composition: 100mM MES, 1mM EGTA, 0.5mM MgSO4, 0.75M NaCl, 20mM NaF

APPLICATIONS

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

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

2D-Xtract™

A protein solubilization buffer for 2D analysis must solubilize proteins effectively, without disturbing the native charge of the proteins. 2D-Xtract™ is 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.

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™ Proteome Kits

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.



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

- Raza, W. et al (2016) Volatile organic compounds produced by Pseudomonas fluorescens WR-1
 restrict the growth and virulence traits of Ralstonia solanacearum. Microbiol. Res. 192:103
- Raza, W. et al (2016) Response of tomato wilt pathogen Ralstonia solanacearum to the volatile organic compounds produced by a biocontrol strain Bacillus amyloliquefaciens SQR-9.Sci Rep. doi:10.1038/srep24856.
- Liu, Y. et al (2016) Growth, microcystin-production and proteomic responses of Microcystis aeruginosa under long-term exposure to amoxicillin. Water Res. 93:141

Cat. No.	Description	Size
<u>786-246</u>	<u>FOCUS</u> [™] <u>Mammalian Proteome</u>	50 preps
<u>786-360</u>	<u>FOCUS</u> <u>Insect Proteome</u>	50 preps
<u>786-257</u>	<u>FOCUS</u> [™] <u>Yeast Proteome</u>	50 preps
<u>786-258</u>	<u>FOCUS</u> <u>Bacterial Proteome</u>	50 preps
<u>786-259</u>	FOCUS [™] Plant Proteome	25 preps

OTHER LYSIS KITS & BUFFERS

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. Based on optimized concentration of Tris and SDS and is suitable for running denaturing electrophoresis and other downstream applications.

CITED REFERENCES

- 1. Zhu, Guang-Fa et al (2015) Exp Ther Med. 1899:0
- 2. Padaria, J.C. et al (2014) BMC Research Notes. 7:713
- 3. Mina, U. et al (2014) Emerging Issues and Challenges. 3:299
- 4. Liu, Y. et al (2011) Lipids Health Dis. 10:117
- 5. Prathyumnan, S. et al Int. J. Cur. Sci. Res. 3:120

Cat. No.	Description	Size
<u>786-225</u>	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.

CITED REFERENCES

- Sharma, S. S. and Mujumdar, S.S. (2017) Transcriptional co-activator YAP regulates cAMP signaling in Sertoli cells. Mol Cell Endocrinol. https://doi.org/10.1016/j.mce.2017.04.017
- Mandal, K. et al (2017) An integrated transcriptomics-guided genome-wide promoter analysis
 and next-generation proteomics approach to mine factor(s) regulating cellular differentiation.
 DOI: https://doi.org/10.1093/dnares/dsw057
- Carbrera, A.P. et al (2016) Senescence Increases Choroidal Endothelial Stiffness and Susceptibility to Complement Injury: Implications for Choriocapillaris Loss in AMD. IOVS doi:10.1167/ioxs.16-19727
- Boakye, C.H.A. et al (2016) Novel amphiphilic lipid augments the co-delivery of erlotinib and IL36 siRNA into the skin for psoriasis treatment. J Control Release. doi:10.1016/j.jconrel.2016.05.017
- Vlaminck, J. et al (2016) Community Rates of IgG4 Antibodies to Ascaris Haemoglobin Reflect Changes in Community Egg Loads Following Mass Drug Administration. PLoS Negl Trop Dis. doi. org/10.1371/journal.pntd.0004532

Cat. No.	Description	Size
<u>786-489</u>	RIPA Lysis & Extraction Buffer	100ml
<u>786-490</u>	RIPA Lysis & Extraction Buffer	500ml
<u>786-723</u>	RIPA Lysis & Extraction Buffer	1L
786-746	RIPA Lysis & Extraction Buffer	1Gal

Inclusion Body Solubilization Buffers

The $IBS^{\mathbb{M}}$ buffer is specifically developed for solubilization of inclusion bodies and $IBS-HP^{\mathbb{M}}$ Buffer for the solubilization of inclusion bodies containing highly hydrophobic proteins.

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. Supplied with optional DTT.

CITED REFERENCES

1. Sheikh, A.H. et al (2013) BMC Plant Biol. 13:121 More cited referneces available at www.gbiosciences.com.

Cat. No.	Description	Size
<u>786-183</u>	IBS [™] Buffer Kit	100ml
786-183HP	IBS-HP [™] Buffer Kit	100ml

DETERGENTS

2D-Detergent[™]

Ultra low conductivity & low carbonyl & peroxide contaminants

Detergents are essential for solubilization of proteins and G-Biosciences offers a wide range of non-ionic, ionic and zwitterionic detergents. For more details download "Detergents Handbook & Selection Guide" from our website.

Our 2D-Detergent™ solutions contain reduced peroxides and carbonyl compounds. In addition, the detergents have less than 15µS conductivity. These detergents are offered as 10% aqueous solutions, sealed under inert gas and are suitable for all protein applications, including 2D-electrophoresis. These non-ionic detergents are suitable for isolating membrane-protein complexes. The aldehyde levels are <50µM, the peroxide levels are <10µM and have a conductivity of <15µS.

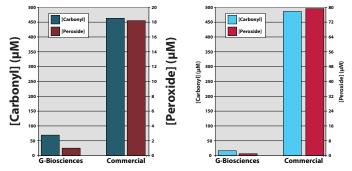


Figure 3: Comparison of carbonyl (blue) and peroxide (red) concentration in G-Biosciences 2D-Detergent™ NP-40 Substitute (left) or 2D-Detergent™ Triton® X-100 (right) and non-proteomic grade commercially available detergents.

Cat. No.	Description	Size
DG907	2D-Detergent [™] Triton [®] X-100	5 x 10ml vials
<u>DG908</u>	2D-Detergent [™] Triton [®] X-100	10 x 10ml vials
<u>DG901</u>	2D-Detergent [™] Nonidet [®] P-40 Substitute	5 x 10ml vials
DG902	2D-Detergent [™] Nonidet [®] P-40 Substitute	10 x 10ml vials

PHOSPHATASE INHIBITOR COCKTAILS

The PhosphataseArrest $^{\mathsf{m}}$ phosphatase inhibitor cocktails are ready-to-use 100X solutions that are simply added to your extraction buffers or samples.

FEATURES

- Single 100X solution
- · Ready-to-use
- · Compatible with most phosphatase assays
- · No resuspension required

CITED REFERENCES

- Bergan-Roller, H.E., et al (2017) Insulin and insulin-like growth factor-1 modulate the lipolytic action of growth hormone by altering signal pathway linkages. Gen Comp Endocrinol. http://doi. org/10.1016/j.ygcen.2017.04.005
- Haley, E. et al (2017) Acidic pH with coordinated reduction of basic fibroblast growth factor maintains the glioblastoma stem cell-like phenotype in vitro. J Biosci Bioeng. http://dx.doi. org/10.1016/j.jbiosc.2016.12.006.
- Mobley C.B. et all (2016) Whey protein-derived exosomes increase protein synthesis and hypertrophy in C2C12 myotubes. Journal of Dairy Science. Doi 10.3168/jds.2016-11341
- Sharp, M.H. et al (2016) The Effects of Fortetropin Supplementation on Body Composition, Strength, and Power in Humans and Mechanism of Action in a Rodent Model. J. Am. Coll. Nutr. doi/10.1080/07315724.2016.1142403
- Durand, S. et al (2016) Hyperphosphorylation amplifies UPF1 activity to resolve stalls in nonsense-mediated mRNA decay. Nat. Commun. doi:10.1038/ncomms12434
- "Wei, D. et al (2016) Inhibiting cortical protein kinase a in spinal cord injured rats enhances efficacy of rehabilitative training. Exp. Neurol. 283:365.
- Martin, J.S. et al (2016) Impact of external pneumatic compression target inflation pressure on transcriptomewide RNA expression in skeletal muscle. Physiol. Rep. DOI: 10.14814/phy2.13029
- Hsieh, C. et al (2016) Persistent increased PKM\(\zeta\) in long-term and remote spatial memory. Neurobiol Learn Mem. DOI: 10.1016/j.nlm.2016.07.009

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^{\mathbb{M}} I is a stablized solution of sodium fluoride, sodium orthovanadate, sodium pyrophosphate, β -glycerophosphate & sodium molybdate.

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.

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.

Cat. No.	Description	Size
<u>786-450</u>	PhosphataseArrest [™] I [100X]	1ml
786-647	PhosphataseArrest [™] I [100X]	24 x 100ul
<u>786-782</u>	PhosphataseArrest [™] I [100X]	2ml
<u>786-783</u>	PhosphataseArrest [™] I [100X]	5ml
<u>786-784</u>	PhosphataseArrest [™] I [100X]	10ml
<u>786-451</u>	PhosphataseArrest [™] II [100X]	1ml
786-452	PhosphataseArrest [™] III [100X]	1ml

PROTEASE INHIBITOR COCKTAILS

For our complete range of protease inhibitors, download "Protease & Phosphatase Inhibitors & Proteases Handbook & Selection Guide".

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 (Ca²+, Mg²+ or Mn²+) 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 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.

ProteaseArrest™ Outperforms Tablet Cocktails

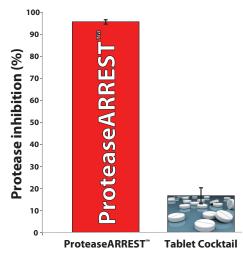


Figure 4: ProteaseArrest™ outperforms tablet format protease inhibitor cocktails. Protease inhibition in mouse pancreas lysate with ProteaseArrest™ (EDTA-free) and a commercially available EDTA-free tablet protease cocktail was compared, using Protease Screening™ Kit. The assay used 0.5mg/ml pancreas lysate and incubation conditions of 37°C for 2.5 hours. ProteaseArrest™ inhibited over 95% of total proteases, 80% more compared to tablet inhibition.

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.

Cell Lysis & Protein Extraction

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 (see figure). 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

CITED REFERENCES

- Abebayehu D. et al (2017) Galectin-1 Promotes an M2 Macrophage Response to Polydioxanone Scaffolds. J Biomed Mater Res A. DOI: 10.1002/jbm.a.36113
- Fletcher, N. et al (2017) Oxidative stress: a key regulator of leiomyoma cell survival. Fertility and Sterility. DOI: http://dx.doi.org/10.1016/j.fertnstert.2017.04.015
- Yu, Q. et al (2017) Comparative proteomics to reveal muscle-specific beef color stability of Holstein cattle during post-mortem storage. Foodchem. 229:769
- Elvington, M. et al (2017) A C3(H20) recycling pathway is a component of the intracellular complement system. J Clin Invest.doi:10.1172/JCl89412.
- Haley, E. et al (2017) Acidic pH with coordinated reduction of basic fibroblast growth factor maintains the glioblastoma stem cell-like phenotype in vitro. J Biosci Bioeng. http://dx.doi. org/10.1016/j.jbiosc.2016.12.006.
- Kim, J.J. et al ((2017) Optical High Content Nanoscopy of Epigenetic Marks Decodes Phenotypic Divergence in Stem Cells. Sci Rep. doi: 10.1038/srep39406
- Yu, Q. et al (2017) Unraveling proteome changes of Holstein beef M. semitendinosus and its relationship to meat discoloration during post-mortem storage analyzed by label-free mass spectrometry. J Proteomics. 154:85

More cited references available at www.gbiosciences.com

Cat. No.	Description	Size
<u>786-108</u>	ProteaseArrest [™] [100X]	2ml
<u>786-437</u>	ProteaseArrest [™] [100X]	5ml
<u>786-711</u>	ProteaseArrest [™] [100X]	10ml
<u>786-712</u>	ProteaseArrest [™] [100X]	5 x 10ml
<u>786-329</u>	<u>OneQuant</u> [™] <u>ProteaseArrest</u> [™] [100X]	24 x 100µl

FOCUS™ ProteaseArrest™

2D electrophoresis & mass spectrometry compatible protease inhibitor cocktail

A ready-to-use, 100X concentrated, broad range protease inhibitor cocktail that is fully compatible with 2D electrophoresis and subsequent mass spectrometry.

The protease inhibitor cocktail contains reversible and irreversible inhibitors of serine, cysteine, calpain and metallo- proteases. Due to the optimized concentration of the various inhibitors, the FOCUS™ ProteaseArrest™ shows excellent inhibition of protease activities and is therefore suitable for the protection of protein samples from animal tissues, plants, yeast and bacteria.

FOCUS™ ProteaseArrest™ is compatible with 2D electrophoresis as it uses an alternative to EDTA as an inhibitor of metalloproteases. The absence of EDTA allows for optimal action of nucleases for removing nucleic acids from the samples. In addition, FOCUS™ ProteaseArrest™ uses PMSF as its primary serine protease inhibitor as opposed to the commonly used Pefabloc®. Pefabloc® has been reported to modify proteins at high concentrations and result in artifacts in subsequent 2D electrophoresis and mass spectrometry.

CITED REFERENCES

- Pier, B. et al (2013) Fertil. Steril. 99:199
- Orkwis, B.R. et al (2010) Genetics 186:885
- Sekar, Y. et al (2010) J Immunol 185:578
- Lupfer, C. and Pastey, M.K. (2010) Virus Res. 149:36
- 5. Zanello, S.B. et al (2006) Curr Eye Res. 21:825

Cat. No.	Description	Size
786-108F	FOCUS [™] ProteaseArrest [™] [100X]	1ml

Protease-PhosphataseArrest™ [100X]

Protease-PhosphataseArrest™ provides full protection of protein samples from proteases and phosphatases released during the preparation of cell and tissue lysates.

Cat. No.	Description	Size
<u>786-870</u>	<u>Protease-PhosphataseArrest</u> [™] _[100X]	For 100ml
<u>786-871</u>	$\underline{\text{Protease-PhosphataseArrest}}\underline{\hspace{0.1cm}}\underline{\hspace{0.1cm}}[100X]$	For 200ml
<u>786-872</u>	Protease-PhosphataseArrest [™] [100X]	For 500ml
786-889	Protease-PhosphataseArrest [™] [100X]	For 240ml

IMMUNOGLOBULIN G REMOVAL

Immobilized Protein A

FEATURES

- High binding capacity: >40mg human IgG/ml resin
- Ligand: Recombinant Staphylococcal Protein A lacking the albumin-binding domain produced in E. coli
- Bead size: 45-165µm
- · Bead Structure: 4% highly cross-linked agarose

CITED REFERENCES

 Kumar, N. et al (2017) Analyzing the role of CagV, a VirB8 homologue of the type IV secretion system of Helicobacter pylori. FEBS Open Bio. DOI: 10.1002/2211-5463.12225

Cat. No.	Description	Size
<u>786-283</u>	Immobilized Protein A Resin	5ml resin
786-824	Immobilized Protein A Resin	25ml resin
<u>786-825</u>	Immobilized Protein A Resin	5 x 1ml columns
<u>786-826</u>	Immobilized Protein A Resin Kit	5 column kit
<u>786-827</u>	Immobilized Protein A Resin	10 x 0.2ml columns
<u>786-828</u>	Immobilized Protein A Resin Kit	10 column kit
<u>786-996</u>	Immobilized Protein A Resin Spin plate	1 plate
<u>786-1031</u>	G-Trap [™] rProtein A FF FPLC Column	2 x 1ml columns
786-1029	G-Trap [™] rProtein A FF FPLC Column	5 x 1ml columns
<u>786-1030</u>	G-Trap [™] rProtein A FF FPLC Column	1 x 5ml columns
786-1032	G-Trap [™] rProtein A FF FPLC Column	5 x 5ml columns
<u>786-902</u>	Immobilized Protein A Magnetic Beads	1ml resin
<u>786-903</u>	Immobilized Protein A Magnetic Beads	5ml resin

Immobilized Protein G

FEATURES

- High binding capacity: 38mg human IgG/ml resin; >20mg sheep IgG/ml resin
- Ligand: Recombinant Streptococcal Protein G lacking the albuminbinding domain produced in E. coli
- Bead size: 50-165µm
- Bead Structure: 4% highly cross-linked agarose

CITED REFERENCES

- Ahmed, S. et al (2017) Stabilization of a soluble, native-like trimeric form of an efficiently cleaved Indian HIV-1 clade C envelope glycoprotein. J Biol Chem.doi: 10.1074/jbc.M117.776419
- Izawa, T. et al (2016) The Nuclear Receptor AhR Controls Bone Homeostasis by Regulating Osteoclast Differentiation via the RANK/c-Fos Signaling Axis. J Immunol doi:10.4049/jimmunol.1600822

Cat. No.	Description	Size
786-829	Immobilized Protein G Resin	2ml resin
786-284	Immobilized Protein G Resin	5ml resin
<u>786-830</u>	Immobilized Protein G Resin	10ml resin
<u>786-831</u>	Immobilized Protein G Resin	25ml resin
<u>786-832</u>	Immobilized Protein G Resin	5 x 1ml columns
<u>786-833</u>	Immobilized Protein G Resin Kit	5 column kit
<u>786-834</u>	Immobilized Protein G Resin	10 x 0.2ml columns
<u>786-835</u>	Immobilized Protein G Resin Kit	10 column kit
<u>786-997</u>	Immobilized Protein G Resin Spin Plate	1 plate
<u>786-1034</u>	G-Trap [™] Protein G FPLC Column	1 x 1ml column
<u>786-1036</u>	G-Trap [™] Protein G FPLC Column	2 x 1ml column
<u>786-1033</u>	G-Trap [™] Protein G FPLC Column	5 x 1ml column
<u>786-1035</u>	G-Trap [™] Protein G FPLC Column	1 x 5ml column
<u>786-1037</u>	G-Trap [™] Protein G FPLC Column	5 x 5ml columns
<u>786-904</u>	Immobilized Protein G Magnetic Beads	1ml resin
<u>786-905</u>	Immobilized Protein G Magnetic Beads	5ml resin

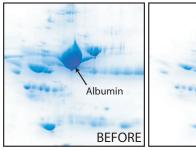
ALBUMIN REMOVAL

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 two dimensional 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. AlbuminOUT™ is suitable for processing 25 or 50 samples.



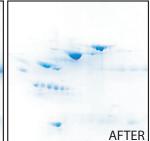


Figure 5: 2D analysis of whole human serum before (left) and after (right) treatment with AlbuminOUT™.

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

- . Shekar, S. et al (2017) 5-LOX in Alzheimer's Disease: Potential Serum Marker and In Vitro Evidences for Rescue of Neurotoxicity by Its Inhibitor YWCS. Mol Neurobiol. DOI: 10.1007/ s12035-017-0527-1
- Sahu, V. Et al (2016) Quantification of Rac1 and Rac1b in serum of non small cell lung cancer by label free real time assay. Clin. Chim. Acta 460: 231
- Kumar, R. et al (2016). Comparative Evaluation of Seven Iso Forms of Serum Sirtuins As Protein Marker for Frailty. Journal of Proteins and Proteomics. 7:101.
- 4. Gupta, A. et al (2015) Biochem Biophys Res Commun. doi:10.1016/j.bbrc.2015.09.181
- 5. Sandilands, E.A. et al (2012) BMC Clin. Pharmacol. 12:3
- 6. De Palma, A. et al (2010) J. Chroma A. 1217:5328

Cat. No.	Description	Size
<u>786-251</u>	<u>AlbuminOUT</u> [™] _	25 preps
786-252	AlbuminOUT™	50 preps

8

Protein Fractionation & Enrichment

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 result 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 fractionation line of products allow for the fractionation of a large selection of biological samples into a multitude of different fractions and these fractions are compatible with a wide range of downstream applications, including 1D & 2D electrophoresis, Western blotting and mass spectrometry.

SPECIFIC PROTEIN ENRICHMENT

Phosphoproteins

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 6: Various concentrations of phosphoprotein were loaded onto the FOCUS $^{\text{\tiny{M}}}$ PhosphoRich $^{\text{\tiny{M}}}$ 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 $^{\text{\tiny{M}}}$.

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
<u>786-255</u>	$\underline{FOCUS}^{\underline{m}}\underline{\ PhosphoRich}^{\underline{m}}$	5 Preps

Glycoproteins

FOCUS^m Glycoprotein kit is based on lectin binding of specific glycoproteins with terminal α -D mannosyl and α -D glycosyl proteins.

FOCUS™ Glycoprotein kit isolates glycoproteins from complex biological solutions using spin columns that contain lectin (Concanavalin A) bound resin with the capacity to bind and immobilize ~5mg glycoproteins. Column bound glycoproteins are eluted with a set of three rapid elution buffers. The proprietary serial elution allows for faster elution compared to other glycoprotein purification systems.

The eluted proteins are suitable for 2D electrophoresis and isoelectric focusing.

FOCUS™ Glycoprotein is designed for 10 x 1.5mg protein samples.

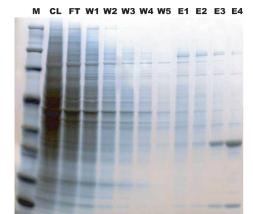


Figure 7: FOCUS™ Glycoprotein isolates multiple glycoproteins. Jurkat cells were lysed by sonication, centrifuged and the supernatant (CL) loaded onto a FOCUS™ Glycoprotein column. The column was centrifuged and the flow through (FT) collected. The column was washed (W1-5) and the glycoproteins were eluted with Glyco Elution Buffer I (E1-2), Glyco Elution Buffer II (E3) and then Glyco Elution Buffer III (E4). 10µI was loaded onto a SDS-PAGE gel, the proteins were resolved and visualized with Reversible Zinc 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

CITED REFERENCES

1. Bhat, R. et al (2015) J. Agric. Food Chem. DOI: 10.1021/acs.jafc.5b04468

Cat. No.	Description	Size
786-253	<u>FOCUS</u> [™] <u>Glycoprotein</u>	10 Preps

Membrane Proteins

For the fractionation of highly enriched 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 cross-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.

Designed for >50 preps, where 1 prep is either 100mg mammalian tissue, 50µl wet animal cell pellet, 50µl wet yeast pellet, 50µl wet bacteria pellet or 250mg plant tissue.

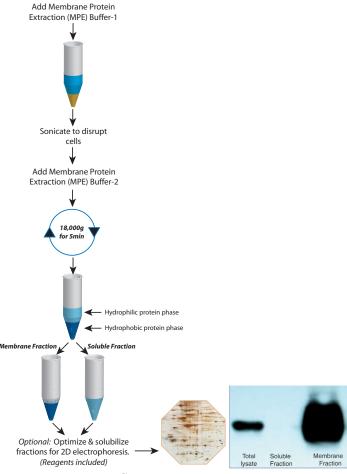


Figure 8: (Left) FOCUS™ Membrane Protein scheme. (Right) Mouse liver was processed with FOCUS™ Membrane Proteins kit. The enriched membrane and soluble fractions were resolved by SDS-PAGE, transferred and probed with antibodies against caveolin, a membrane protein.

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

CITED REFERENCES

- 1. Weigum, S. E. et al (2014) SPIE. doi:10.1117/12.2062283
- 2. Kostyal, D. et al (2012) Med. Oncol. 29:1486
- Peng, H. et al (2007) Cancer Res. 67:9346

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

Cell Surface Protein Isolation

For the biotin labeling and subsequent isolation of cell surface proteins

HOOK™ Cell Surface Protein Isolation kit uses G-Biosciences 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, including Western blotting.

Mammalian cells, adherent or non-adherent, are first labeled with HOOK™ Sulfo-NHS-SS-Biotin. HOOK™ Sulfo-NHS-SS-Biotin is a water-soluble, amine reactive biotinylation reagent that has a N-hydroxysulfosuccinimide (sulfo-NHS) ester. The addition of a charged sulfonate (SO³) on the N-hydroxysuccinimide ring of the sulfo-NHS esters results in its solubility in water, but prevents it permeating plasma membranes. The solubility and impermeability to plasma membranes makes HOOK™ Sulfo-NHS-SS-Biotin ideal for studying cell surface proteins as it will only react with the protein molecules on the outer surface of plasma membranes. An additional advantage of HOOK™ Sulfo-NHS-SS-Biotin is the presence of a disulfide bond in the spacer arm. Disulfide bond permits the cleavage of the biotin from the protein, making its interaction with streptavidin reversible.

Following labeling, cells are lysed with Mammalian Cell PE LB™, a buffered lysis solution that employs a mild non-ionic detergent for enhanced extraction and stability of proteins, and the cell lysate is applied to a Streptavidin agarose column. Unlabeled intracellular

proteins are washed away and the biotin labeled cell surface proteins are then released by reduction 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-75cm2 flasks.

FEATURES

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

QUENCH

CELL LYSIS

Biotin S-S Protein

DTT

Biotin SH HS Protein

Figure 9: HOOK™ Cell Surface Protein Isolation scheme.

- 1. 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
<u>786-316</u>	HOOK [™] Cell Surface Protein Isolation	5 Expts

Signal Proteins

Enrichment of signal proteins in lipid rafts

Lipid rafts are membrane microdomains that are enriched in caveolin, cholesterol, glycolipids, sphingolipids and glycosylphosphatidylinositol. 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 nonionic 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.

Designed for >50 preps, where a prep is either 100mg mammalian tissue, $50\mu l$ wet animal cell pellet, $50\mu l$ wet yeast pellet, $50\mu l$ wet bacteria pellet or 250mg plant tissue

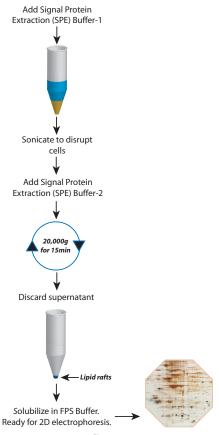


Figure 10: FOCUS™ Signal Protein scheme.

APPLICATIONS

- · Isolate signal proteins that are localized to lipid rafts
- · Study movement of activated proteins to and from lipid rafts
- Suitable for wide range of downstream applications, including 1D & 2D electrophoresis, Western blotting and mass spectrometry

CITED REFERENCES

1. Hsu, E. et al (2015) Neoplasia. 17:497

Cat. No.	Description	Size
<u>786-250</u>	FOCUS [™] Signal Protein	50 Preps

Soluble & Insoluble Proteins

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. Supplied with reagents necessary for fractionation of soluble and insoluble fractions, including a strong chaotropic extraction buffer to solubilize difficult proteins.

Also 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 1 prep is:

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

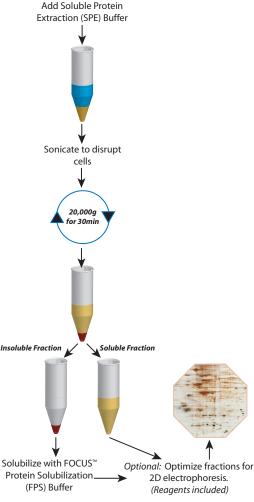


Figure 11: FOCUS™ Soluble & Insoluble scheme.

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

FOCUS™ Global Fractionation

Enrichment of cytosolic and membrane signal, peripheral or integral proteins

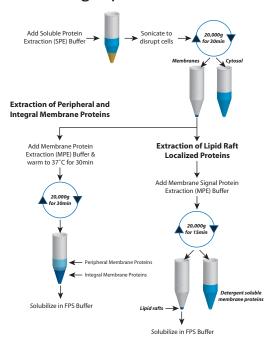


Figure 12: FOCUS™ Global Fractionation scheme.

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 glycosylphosphatidylinositol. Lipid rafts are also known as detergentinsoluble 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.

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

FEATURES

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

APPLICATIONS

- For downstream applications, including 1D & 2D electrophoresis, Western blotting and mass spectrometry
- · Suitable for proteomics and cell signaling studies

CITED REFERENCES

- Wan H. et al (2016) Identification of transporter proteins for PQQ-secretion pathways by transcriptomics and proteomics analysis in Gluconobacter oxydans WSH-003. Front Chem Sci Eng. doi:10.1007/s11705-016-1580-4
- Liu, P.D. et al (2016) Characterization of purple acid phosphatases involved in extracellular dNTP utilization in Stylosanthes. J. Exp. Bot. published 18 May 2016, 10.1093/jxb/erw190
- Harada, K. et al (2016) Biochemical and Biophysical Research Communications. Biochem Biophys Res Commun. 469:993

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

ORGANELLE FRACTIONATION

Nuclear & Cytoplasmic 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 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.

Designed for >50 preps, depending on the tissue used, where one prep is 20x10° mammalian cells or 100mg mammalian tissue.

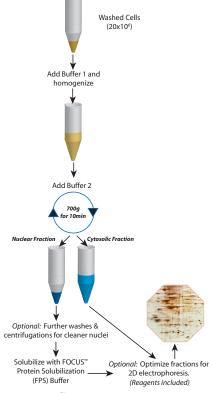


Figure 13: FOCUS™ Cytoplasmic & Nuclear Protein scheme.

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

- 1. Riviere, L. et al (2015) J Hepatol. DOI: http://dx.doi.org/10.1016/j.jhep.2015.06.023
- 2. Kim, Y.H. et al (2007) Neurobiol. Dis. 26:569
- 3. Wang, T. et al (2007) Biochem. Bioph. Res. Co. 352:203
- 4. Kim, Y.H. et al (2007) Life Sciences. 81:1167
- 5. Rehman, A. et al (2005) Breast cancer Res. 7:R765

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

Mitochondrial Proteins

For enrichment of intact, active 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.

Highly adaptable kit for use with animal tissues and other sources of mitochondria. The kit is designed for 50 preps, where one prep is equivalent to 2x10⁷ mammalian cells or 100mg mammalian tissue.

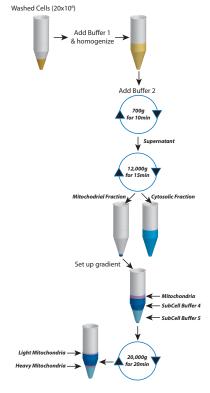


Figure 14: FOCUS™ Mitochondria scheme.

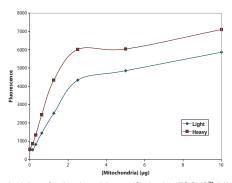


Figure 15: Activity of mitochondria purified with FOCUS™ Mitochondria, as determined with a JC-1 Assay.

FEATURES

- · Fast and efficient fractionation of active heavy & light mitochondria
- Suitable for wide range of downstream applications, including 1D & 2D electrophoresis, Western blotting and mass spectrometry

CITED REFERENCES

- Dagda, R.K. et al (2014) J. Neurochem. 128:864
- Rigobello, M.P. et al (2009) Free Rad. Biol. Med. 47:710

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

Subcellular Fractionation

For the fractionation of mitochondrial, nuclear, and cytosolic soluble and membrane proteins

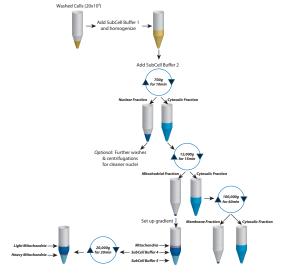


Figure 16: FOCUS™ SubCell scheme.

FOCUS™ SubCell is for the total subcellular fractionation of cells and mammalian tissue into enriched fractions of nuclear, mitochondrial, cytosolic and membrane proteins.

The resulting mitochondrial fraction can be subsequently separated into heavy and light fractions by gradient centrifugation and the resulting mitochondria are >90% active and have intact inner and outer membranes.

The kit includes reagents for optional steps that minimize contaminations of the nuclear fraction by cytoplasmic elements.

Suitable for cultured animal cells and adaptable for animal tissues. Designed for 50 preps, where one prep is equivalent to 2x107 mammalian cells or 100mg mammalian tissue.

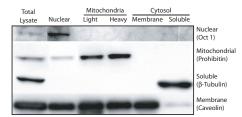


Figure 17: NIH3T3 cells were fractionated with FOCUS™ SubCell. The fractions were resolved by SDS-PAGE and then transferred to a PVDF membrane. The membrane was probed with the indicated antibodies.

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

- Shiheido, Y. et al (2016) Porphyromonas gingivalis, a periodontal pathogen, enhances myocardial vulnerability, thereby promoting post-infarct cardiac rupture.J Mol Cell Cardiol. DOI: http:// dx.doi.org/10.1016/j.yjmcc.2016.03.017
- Das, S. et al (2015) Sci Rep doi:10.1038/srep09759
- Capel, F. et al (2015) J. Nutr. Biochem. doi:10.1016/j.jnutbio.2015.04.003
- Dagda, R.K. et al (2014) J. Neurochem, 128:864 3.
- 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

Cat. No.	Description	Size
<u>786-260</u>	<u>FOCUS</u> [™] <u>SubCell Kit</u>	50 Preps

Protein Fractionation & Enrichment

GENERAL FRACTIONATION

Fraction-FOCUS™

Fractionation for cleaner 2D gel maps

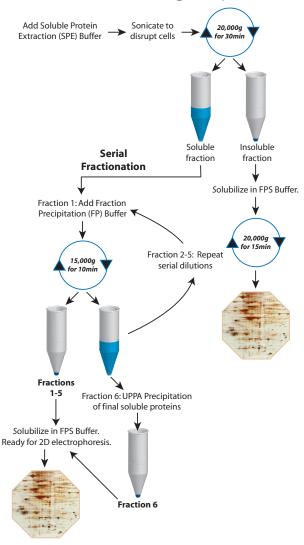


Figure 18: Fraction-FOCUS™ scheme.

2D electrophoresis and mass spectrometry are 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 total protein during the Fraction-FOCUS $^{\text{M}}$ procedure. At the end of the fractionation, cellular proteins are in one of many fractions.

Designed for 10 preparations, where one preparation is either 100mg mammalian tissue, 50µl wet animal cell pellet, 50µl wet yeast pellet, 50µl wet bacteria pellet or 250mg plant tissue

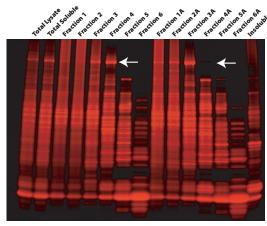


Figure 19: Mouse liver lysate was analyzed with Fraction-FOCUS™ (Fractions1-6, normal protocol; Fractions 1A-6A, alternative protocol). 5µg protein was loaded on a 4-20% SDS polyacrylamide gel and stained with a fluorescent stain. Fraction-FOCUS™ simplifies the protein maps, compared to total lysate, and a simple change in the protocol generates a different protein profile and allows proteins to be concentrated in the same fractions (see arrows). Protein resolution will be greatly improved when fractions are visualized by 2D electrophoresis.

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
<u>786-168</u>	Fraction-FOCUS [™]	10 Preps

Clean-up & Concentration

DETERGENT REMOVAL SYSTEMS

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®.

The Detergent-OUT™ products are suitable for removing detergent from all types of protein solutions, including hydrophobic protein solutions.

DetergentOUT™ GBS10

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.

DetergentOUT™ GBS10 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.

In independent studies DetergentOUT[™] GBS10 was shown 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.

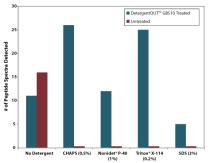


Figure 20: DetergentOUT $^{\infty}$ GBS10 removes detergent and allows detection of peptide fragments by Mass spectrometry. 500µg phosphorylase B was digested in solution and then the indicated amount of detergent was added. Samples were treated with DetergentOUT $^{\infty}$ GBS10. Number of peptide spectra were determined as per the protocol of Alvarez, S. et al.

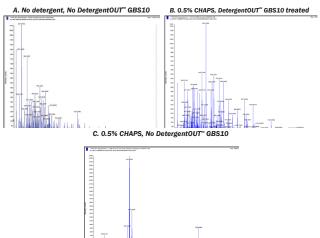


Figure 21: DetergentOUT GBS10 enhances Mass spectrometry Spectra. $5\mu g/\mu l$ protein mixture (BSA, cyctochrome C and phosphorylase B) in water (Panel A) was supplemented with 0.5% CHAPS (Panel B and C). The CHAPS containing sample was treated with DetergentOUT GBS10 and compared to an untreated sample (Panel C). Spectra were generated per Alvarez et al.

	%		Phosphorylase	Cytochrome	E.coli
Detergent	Removed	BSA	В	С	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.

CITED REFERENCES

- Haddock, E. and Feldmann, F. (2017) Validating the Inactivation Effectiveness of Chemicals on Ebola Virus. Methods Mol Biol. 1628:251
- Srivastasva, O.P. et al (2017) Post-Translationally Modified Human Lens Crystallin Fragments Show Aggregation in vitro. Biochemistry and Biophysics Report. http://dx.doi.org/10.1016/j. bbrep.2017.01.011
- Chiu, J. et al (2016) Knockout of a difficult-to-remove CHO host cell protein, lipoprotein lipase, for improved polysorbate stability in monoclonal antibody formulations. Biotechnol Bioeng. DOI: 10.1002/bit.26237

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

OrgoSol DetergentOUT™

Suitable for hydrophobic proteins

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 detergent concentration in the solution, is highly flexible and can 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.

CITED REFERENCES

- 1. Cortes, D.F. et al (2012) Electrophoresis. 33:3712
- Orr, S.J. et al (2012) Molecular Sys Biol. 8:573
 Troese, M.J. et al (2011) Infect Immunol. 79:4696

Cat. No.	Description	Size
<u>786-127</u>	OrgoSol DetergentOUT [™] , Micro	For 10ml
<u>786-128</u>	OrgoSol DetergentOUT [™] , Medi	For 30ml

DetergentOUT™ Tween®

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

Cat. No.	Description	Size
<u>786-214</u>	<u>DetergentOUT</u> [™] <u>Tween</u> [®] , <u>Micro</u>	10 columns
786-215	<u>DetergentOUT</u> <u>Tween®, Medi</u>	10 columns

Clean-up & Concentration

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 $^{\mathbb{N}}$ allows for easy handling and manipulation. For sample recovery, just place the Tube-O-DIALYZER $^{\mathbb{N}}$ 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 $^{\mathbb{M}}$ 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 $^{\mathbb{M}}$ 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 $^{\mathbb{M}}$, 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

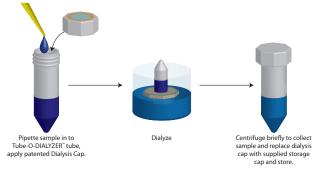


Figure 22: A summary of the Tube-O-DIALYZER $^{\text{\tiny{M}}}$ system.

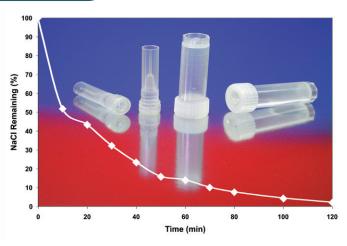


Figure 23: Tube-O-DIALYZER™ micro (8K MWCO) Dialysis Rate. 100µl 5M sodium chloride was dialyzed against 1 liter deionized water. 50% sodium chloride is removed in the first 10 minutes.

- Aslanyan, L. et al (2017) Effect of Urea on G-Quadruplex Stability. J. Phys. Chem. B. DOI: 10.1021/acs.jpcb.7b03479
- Han, S. et al (2017)Fluorescent nanoswitch for monitoring specific pluripotency-related microRNAs of induced pluripotent stem cells: Development of polyethyleneimineoligonucleotide hybridization probes. Nano Res. doi:10.1007/s12274-016-1403-4
- Kukreja, A et al (2016) Preparation of gold core-mesoporous iron-oxide shell nanoparticles and their application as dual MR/CT contrast agent in human gastric cancer cells. Journal of industrial and Engineering Chemistry. doi.org/10.1016/j.jiec.2016.12.020
 Xue, H.Y et al (2016) A biodistribution study of solid lipid-polyethyleneimine hybrid nanocarrier
- Xue, H.Y et al (2016) A biodistribution study of solid lipid-polyethyleneimine hybrid nanocarries for cancer RNAi therapy. Eur J Pharm Biopharm 108:68
- Kim, B.G and Chalikian T.V (2016) Thermodynamic linkage analysis of pH-induced folding and unfolding transitions of i-motifs. Biophys Chem. Vol 216:19
- Barnaba, C. et al (2016) Substrate Dependent Native Luminescence From Cytochromes P450 3A4, 2C9, and P450cam. J. Phys. Chem. B.DOI: 10.1021/acs.jpcb.5b11804
- 7. Marin-Argany, M. et al (2015) Protein Science. DOI: 10.1002/pro.2790
- Tarasava, K. and Freisinger, E. (2015) J Inorg Biochem doi:10.1016/j.jinorgbio.2015.08.009
 Samejima, K. et al (2015) J Biol Chem 290:21460
- 10. Gu, R. Et al (2015) Colloids and Surfaces B: Biointerfaces. 135:126
- Byul, K.G. et al (2015) Biochem. 54:3420
 Kim, H. J. et al (2015) Biomaterials. 61:95
- More cited references available at www.gbiosciences.com

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

CONTAMINATION REMOVAL

Spin-OUT™ Desalting Columns

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 peptide, protein and nucleic acid solutions ranging from 5µl to 4ml sample volumes. Also available in 96-well spin plate formats for processing up to 96 samples.

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.

The SpinOUT™ columns are 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 SpinOUT™ GT-100, <1,000Da for SpinOUT™ GT-600 and <1,500Da for SpinOUT™ GT-1200).

Spin-OUT™ GT-100 is for the purification of peptides and proteins >700Da.

Spin-OUT™ GT-600 is 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.

CITED REFERENCES

- Yang, Y. et al (2016) PROCEEDINGS, 41st Workshop on Geothermal Reservoir Engineering. Stanford University, Stanford, California
- Wang, X. et al (2015) Zoolog Sci. 32:419
- Fedosejevs, E.T et al (2014) J Biol Chem. 289: 33412 Wickremasinghe, N. C. et al (2014) Biomacromolecules. 15:3587
- Shane, M.W. et al (2013) Plant Physiol. 161:1634
- Vitrac, H. et al (2013) PNAS 110:9338
- Singh, J. et al (2012) Gastroenterology. 143:1308
- Singh, J. et al (2009) Am. Physiol.-Gastr. L. 297:G1206
- Gibbons, A.M. et al (2009) J. Microencaps. 26:513. Cryan, S. et al (2006) Mol. Pharm. 3:104
- Tripodi, K. et al (2005) Plant Physiol 139:969
- Taggart, C. et al (2005) J Exp Med 202:1659

Cat. No.	Description	Size	Resin Bed (ml)	Sample Load (ml)
<u>786-865</u>	SpinOUT [™] GT-100, 0.1ml	25 columns	0.1	0.005-0.02
<u>786-866</u>	SpinOUT [™] GT-100, 1ml	10 columns	1	0.05-0.1
<u>786-867</u>	SpinOUT [™] GT-100, 3ml	10 columns	3	0.1-0.5
<u>786-897</u>	SpinOUT [™] GT-100, 3ml	5 x 10/bag	3	0.1-0.5
<u>786-868</u>	SpinOUT [™] GT-100, 5ml	5 columns	5	0.5-2
<u>786-898</u>	SpinOUT [™] GT-100, 5ml	50 columns	5	0.5-2
<u>786-869</u>	SpinOUT [™] GT-100, 10ml	5 columns	10	0.5-4
<u>786-703</u>	SpinOUT [™] GT-600, 0.1ml	25 columns	0.1	0.005-0.02
<u>786-170</u>	SpinOUT [™] GT-600, 1ml	10 Columns	1	0.02-0.13
786-720	SpinOUT [™] GT-600, 1ml	5 x 10/bag	1	0.02-0.13
<u>786-171</u>	SpinOUT [™] GT-600, 3ml	10 Columns	3	0.1-0.5
<u>786-721</u>	SpinOUT [™] GT-600, 3ml	5 x 10/bag	3	0.1-0.5
<u>786-704</u>	SpinOUT [™] GT-600, 5ml	5 columns	5	0.5-2
<u>786-722</u>	SpinOUT [™] GT-600, 5ml	50 columns	5	0.5-2
<u>786-705</u>	SpinOUT [™] GT-600, 10ml	5 columns	10	0.5-4
<u>786-989</u>	SpinOut [™] GT-600 Spin Plate	2 plates	1	0.02-0.13
<u>786-990</u>	SpinOut [™] GT-600 Spin Plate	4 plates	1	0.02-0.13
<u>786-706</u>	<u>SpinOUT</u> [™] <u>GT-1200, 0.1ml</u>	25 columns	0.1	0.005-0.02
<u>786-172</u>	SpinOUT [™] GT-1200, 1ml	10 Columns	1	0.05-0.1
<u>786-173</u>	SpinOUT [™] GT-1200, 3ml	10 Columns	3	0.1-0.5
<u>786-707</u>	SpinOUT [™] GT-1200, 5ml	5 columns	5	0.5-2
<u>786-708</u>	<u>SpinOUT</u> GT-1200, 10ml	5 columns	10	0.5-4
<u>786-991</u>	SpinOut [™] GT-1200 Spin Plate	2 plates	1	0.02-0.13
<u>786-992</u>	SpinOut [™] GT-1200 Spin Plate	4 plates	1	0.02-0.13

CONCENTRATION SYSTEMS

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.

Available for concentrating up to 10ml or 30ml of protein solutions. UPPA™ (Universal Protein Precipitation Agent) is offered separately for the concentration of dilute (>1ng/ml) protein solutions.

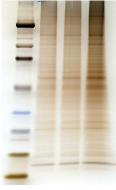


Figure 24: Concentration of dilute mouse liver lysate. Lane 1: Protein Marker; Lane 2: 20µl dilute protein (0.05µg/µl). Lane 3: 20µl original sample treated with UPPA-PROTEIN-Concentrate™ and resuspended in 20µl. Lane 4: 40µl original sample treated with UPPA-PROTEIN-Concentrate™ and resuspended in 20µl. Comparing lanes 2 and 3 shows that there is 100% protein recovery and lane 4 shows the actual concentration of a sample.

CITED REFERENCES

- Wan, C. et al (2015) Data Brief. doi:10.1016/j.dib.2015.11.062
- Scherp, P. et al (2011) Methds Mol. Biol. 702:163
- McFadden, N. et al (2011) PLoS Pathog 7(12): e1002413
 - Grote, J. et al (2006) BMC Mol. Biol. 7:48
 - 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	$\underline{UPPA\text{-}PROTEIN\text{-}Concentrate^{\underline{m}}\underline{(Micro)}}$	For 10ml sample
<u>786-121</u>	<u>UPPA-PROTEIN-Concentrate[™] (Medi)</u>	For 30ml sample
786-122	<u>UPPA</u> [™] -I & II Pack	For >80ml

OrgoSol-PROTEIN-Concentrate™

Preserve biological activity during concentration

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.

- 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
<u>786-125</u>	<u>OrgoSol-PROTEIN-Concentrate</u> ™	For 5ml protein

Clean-up & Concentration

Column-PROTEIN-Concentrate™

For larger volumes of dilute protein solutions

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. 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.

CITED REFERENCES

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

Cat. No.	Description	Size
<u>786-126</u>	Column-PROTEIN-Concentrate [™]	For 4mg protein

Tube-O-CONCENTRATOR™

Rapid concentration 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.

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

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

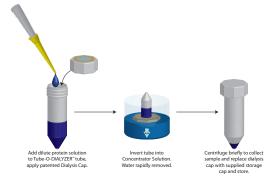


Figure 25: Tube-O-CONCENTRATOR $^{\text{™}}$ scheme.

CITED REFERENCES

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

Cat. No.	Description	Size
<u>786-625</u>	Tube-O-CONCENTRATOR [™] for 20-250μl	5 concentrations
<u>786-626</u>	Tube-O-CONCENTRATOR [™] for 0.2-2.5ml	5 concentrations

ELECTROPHORESIS CLEAN UP

PAGE-Perfect[™]

Improved resolution & publication quality gels

Many lysis buffers and reagents are incompatible with routinely used electrophoretic analysis. The presence of 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 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 all interfering agents. Suspend the precipitate in the sample loading buffer for loading on the gel for electrophoresis. The figure demonstrates the effect of PAGE-Perfect™ on the clean-up of 10µg mouse liver lysate that contain the indicated contaminants.

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

- 1. Devillard, E. et al (2004) J Bacteriol 186:136
- 2. 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
- 5. Grimaldi, M. et al (2003) J Neurosci 23:4737
- 6. Wu, X. et al (2002) JBC 277:13597



Figure 26: 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 a SDS polyacrylamide gel. B. 10µg mouse liver lysates, in the presence of various interfering agents, were treated with PAGE-Perfect[™] and then loaded onto a SDS polyacrylamide gel. Both gels were stained with FASTsilver[™] protein.

Cat. No.	Description	Size
<u>786-123</u>	<u>PAGE-Perfect</u> [™]	50 preps
<u>786-123T</u>	<u>PAGE-Perfect</u> [™]	5 preps

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 and shows identical peptide spectra.

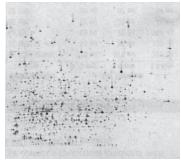


Figure 27: Treatment of protein samples with Perfect-FOCUS™ results in streak free, high spot resolution gels.

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

- Liu, Y. et al (2016) Growth, microcystin-production and proteomic responses of Microcystis aeruginosa under long-term exposure to amoxicillin. Water Res. 93:141
- 2. Bhuyan, S.K. et al (2016) Extraction of proteins for twodimensional gel electrophoresis and proteomic analysis from an endophytic fungus. Protocol Exchange
- Ghosal, K. et al (2016) AACIJournal, DOI: 10.1186/s13223-016-0135-z
- Nardi, J.B. et al (2016) Segmental pairs of giant insect cells discharge presumptive immune proteins at each larval molt. Dev Biol.doi:10.1016/j.ydbio.2016.03.029
- Dey, D. et al (2016) Curvularia pallescens, a prevalent aerospore in India. Biochim. Biophys. Acta.doi:10.1016/j.bbapap.2016.03.012 Zhang, J. et al (2015) Int J Coal Geo. 146:91
- Kanaujia, P.K. et al (2015) J Proteomics. 124:39 6.
- Chen, W. et al (2014) Acta Physiol Plant 37:1723
- Freiberg, J. A. et al (2014) Infect and Immun. doi: 10.1128/IAI.01831-14
- Frapsauce, C. et al (2014) Fertil Steril. http://dx.doi.org/10.1016/j.fertnstert.2014.04.039
- Kerns, P.W. et al (2014) Pathogens and Disease. DOI: 10.1111/2049-632X.12142 10.
- Kokjohn, T.A. et al (2013) J. Neurotraum. 30:981
- 12 Solazzo, C. et al (2013) Int. Biodeter, Biodegrad, 80:48
- Fekkar, A. et al (2012) J. Infect Dis. 205:1163 13.
- Shen, J. et al (2012) Carcinogenesis. 33:2208
- Gandaharen, Y.D. et al (2012) Modulation of HER2 Tyrosine/Threonine Phosphorylation and Cell Signalling, Protein Phosphorylation in Human HealthDOI: 10.5772/50472. Wang, H. et al (2012) Method Mol. Biol. 876:83
- 16.
- Walseth, T.F. et al (2012) Messenger. 1:86

Cat. No.	Description	Size
<u>786-124</u>	<u>Perfect-FOCUS</u> [™]	50 preps
786-124T	Perfect-FOCUS [™]	6 preps

PROTEIN MARKERS

PAGEmark[™] Blue PLUS

A blue protein standard with 12 prestained proteins covering a wide range of molecular weights:

- 10-240kDa in Tris-Glycine buffer
- 9-235kDa in Bis-Tris (MOPS) buffer or Bis-Tris (MES) buffer

Proteins are covalently coupled with a blue chromophore and two reference bands, 25kDa and 72kDa, are enhanced in intensity for easier reference. Use 3-5µl/well on mini gels for clear visualization during electrophoresis or 1.2-2.5µl per well for Western transfers.

Color	Tris-	Bis-Tris	Bis-Tris
	Glycine	(MOPS)	(MES)
	240	235	235
	180	170	170
	140	130	130
	100	93	93
	72	68	70
Dluc	60	53	53
Blue	45	41	42
	35	30	30
	25	22	23
	20	18	18
	15	14	14
	10	9	9

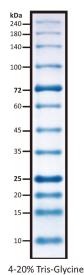


Figure 28: PAGEmark™ Blue PLUSmarkers.

PAGEmark™ Tricolor PLUS

A ready-to-use three-color protein standard with 13 prestained proteins covering a wide range of molecular weights:

- 5-245kDa in Tris-Glycine buffer
- 3.5-235kDa in Bis-Tris (MOPS) buffer or Bis-Tris (MES) buffer Proteins are covalently coupled with a blue chromophore except for two reference bands. A green at 25kDa and a red at 75kDa. Use 3-5µl/well on mini gels for clear visualization during electrophoresis

or 1.2-2.5ul per well for Western transfers

or 1.2-2.5µr per weir for western transfers.			
Color	Tris- Glycine	Bis-Tris (MOPS)	Bis-Tris (MES)
Blue	245	235	235
Blue	180	170	170
Blue	140	130	130
Blue	100	93	93
Red	75	70	72
Blue	60	53	53
Blue	45	41	42
Blue	35	30	30
Green	25	22	23
Blue	20	18	18
Blue	15	14	14
Blue	10	9	10
Dive		2.5	2 -

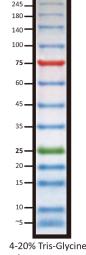


Figure 29: PAGEmark™ TriColor PLUSmarkers.

Cat. No.	Description	Size
<u>786-418</u>	PAGEmark™ Blue PLUS Marker	100 loads at 5µl/well
786-419	PAGEmark™ Tricolor PLUS Marker	100 loads at 5µl/well

PROTEIN REDUCTION & ALKYLATION

FOCUS™ Protein Alkylation

The kit is supplied with a proprietary buffer necessary for efficient alkylation of thiols, while minimizing reoxidation of the competing thiol pairs in protein samples. Simply add an appropriate amount to reagent solutions for alkylation of protein thiol groups.

APPLICATIONS

- Preparation of protein samples for 2D electrophoresis
- · Blocking of thiols for preventing re-oxidation of proteins

Cat. No.	Description	Size
786-232	<u>FOCUS</u> <u>Protein Alkylation Kit</u>	100 preps

FOCUS™ Protein Reduction-Alkylation

Improves resolution and prevents streaking

A simple two-step method for reduction and alkylation of protein samples for 2D gel analysis. The disulfide bonds are reduced with a highly reactive and stable TCEP [Tris (2-carboxyethyl) phosphine] followed by blocking of the thiols by alkylation with iodoacetamide reagent preparation.

The kit is supplied with proprietary buffers and reagents necessary for an efficient reduction and alkylation of the disulfide bridges while minimizing reoxidation of the thiols.

FEATURES

- 2D electrophoresis compatible
- · Reduction & alkylation of protein samples in two simple steps
- Minimizes reoxidation of the competing thiol pairs

CITED REFERENCES

- 1. Wilks, A.M. et al (2015) J. Exp. Biol. 218:3128
- 2. Carter, M.Q. et al (2012) Appl. Envir. Microbiol. 78:1004
- Fagerquist, C.K. (2012) Rapid Commun. Mass Sp. 26:1241
 Järvinen, K.M. et al (2012) Clin. Exper. Allergy. 42:238

Cat. No.	Description	Size
786-231	FOCUS™ Protein Reduction-Alkylation	100 preps

FOCUS™ Protein Reductant

A water soluble, odorless, non-toxic and stable TCEP [Tris (2-carboxyethyl) phosphine] reductant for protein reduction.

FOCUS™ Protein Reductant has improved stability and efficiency compared to DTT and reduces proteins over a wide range of pH, including lower acidic pH. FOCUS™ Protein Reductant completely reduces highly stable disulfide bonds in less than 5 minutes at room temperature.

FOCUS™ Protein Reductant is compatible with the alkylation of thiol groups for 2D analysis, as it does not compete with the alkylation reagent iodoacetamide, unlike DTT and other commonly used reductants.

The kit is supplied with a proprietary buffer necessary for efficient reduction of disulfide bonds, while minimizing reoxidation of the competing thiol pairs in protein samples.

FEATURES

- A water soluble, odorless, non-toxic and stable reducing agent
- Reduces disulfides and minimizes re-oxidation of the competing thiol pairs in protein samples

Cat. No.	Description	Size
786-230	FOCUS™ Protein Reductant	100 preps

FOCUS™ DTT [2M]

Ultra pure proteomic grade DTT for protein reduction. FOCUS™ DTT is supplied as 5 vials containing dry powder to maintain the activity of the reducing agent. Add 1ml aliquot of water to give a 2M concentration, which is then added to your buffer solution to maintain protein reduction potential.

Cat. No.	Description	Size
786-227	FOCUS [™] DTT [2M]	5 x 1ml

lodoacetamide

lodoacetamide is supplied in 5 gram bulk quantities or in the $OneQuant^{\mathbb{M}}$ format. Add 150µl water to a single $OneQuant^{\mathbb{M}}$ vial to generate a 0.5M iodoacetamide solution.

CITED REFERENCES

- Bora de Oliveira, K. et al (2017) Site-specific monitoring of N-glycosylation profiles of a CTLA4-Fcfusion protein from the secretory pathway to the extracellular environment. Biotechnol Bioeng. DOI: 10.1002/bit.26266
- Provan, F. et al (2016) An evaluation of coral lophelia pertusa mucus as an analytical matrix for environmental monitoring: A preliminary proteomic study. J Toxicol Environ Health A doi.org/10.1 080/15287394.2016.1210494
- 3. Thompson, J.H. et al (2014) Rapid Comm. Mass Spec. 28:855
- Moharram, R. et al (2006) FEBs Let. 580:3391

Cat. No.	Description	Size
<u>786-228</u>	lodoacetamide	5g
RC-150	lodoacetamide	25g
<u>786-078</u>	<u>OneQuant</u> <u>Iodoacetamide</u>	40 vials

2D ELECTROPHORESIS ACCESSORIES

Agarose Sealing Solution

For sealing IPG strips while running 2D gels

Simply heat and use for sealing the IPG strips for SDS-PAGE analysis. The Agarose Sealing Solution is prepared in a proprietary buffer to minimize reoxidation of the competing thiol pairs as proteins enter into the second dimension gel. Improves resolution and prevents streaking of protein spots on 2D gels.

Cat.	No.	Description	Size
786-	226	Agarose Sealing Solution	50ml

Equilibration Buffer for IPG Strips

Add an appropriate volume of the supplied rehydration buffer to the dry, ready-to-use, urea based equilibration buffer. Eliminates worries about urea induced protein carbamylation or waste of unused reagents, as fresh and ready-to-use reagents are prepared every time.

Supplied with a proprietary buffer necessary to support efficient reduction of the disulfide bridges and alkylation of the thiols, while minimizing reoxidation of the competing thiol pairs in protein samples. Improves resolution and prevents streaking of protein spots on 2D gels.

CITED REFERENCES

1. Brasier, A. et al (2004) J Virol 78:11461

Cat. No.	Description	Size
<u>786-224</u>	Equilibration Buffer for IPG Strips	For 50ml

Proteomic Grade Water

Ultra-pure water for 2D and mass spectrometry analysis.

Cat. No.	Description	Size
786-229	Proteomic Grade Water	1L

Proteomic Grade Tubes

Specifically prepared and certified protein and dust free.

Cat. No.	Description	Size
786-300	Proteomic Grade Microfuge Tubes (1.5ml)	100

Proteomic Protein Controls

Protein preparations specifically prepared for standardizing electrophoresis methods and protocols.

Substantially free from non-protein agents such as nucleic acids, detergents, salts, lipids and other common laboratory agents. The protein preparation has low conductivity (< 50μ S). Supplied as dry protein pellets (2 x 2mg/vial).

A control set containing one 2mg vial of mammalian, E. coli, yeast and plant proteins is also offered.

CITED REFERENCES

 Chang, W.W.P. et al (2005) Enhanced Resolution Achieved With Electroosmotic Flow Control in Capillary Isoelectric Focusing With Dynamic Coatings Amer. Biotech. Lab

Cat. No.	Description	Size
PSC-01	Proteomic Protein Control, Yeast	2 x 2mg
PSC-02	Proteomic Protein Control, Animal cells	2 x 2mg
PSC-03	Proteomic Protein Control, E. coli	2 x 2mg
PSC-04	Proteomic Protein Control, Plant	2 x 2mg
PSC-05	Proteomic Protein Control Set	4 x 2mg

Electrophoresis Buffers & Reagents

0 1 N	B :::	0'
Cat. No.	Description	Size
<u>786-502</u>	Acrylamide/ Bisacrylamide (37.5:1); 40% Solution 38.96% solution containing acrylamide (40%) and bisacrylamide (1.04%) for cross-linker ratio of 37.5:1	500ml
<u>786-503</u>	Acrylamide/ Bisacrylamide (37.5:1); Premixed powder	40g
<u>786-504</u>	Acrylamide/ Bisacrylamide (37.5:1); Premixed powder	200g
<u>786-505</u>	Acrylamide/ Bisacrylamide (29:1): 40% Solution 40% solution containing acrylamide (38.67%) and bisacrylamide (1.33%) for cross-linker ratio of 29:1	500ml
<u>786-506</u>	Acrylamide/ Bisacrylamide (29:1); Premixed powder	40g
<u>786-507</u>	Acrylamide/ Bisacrylamide (29:1); Premixed powder	200g
RC-001	Acrylamide Powder	100g
RC-002	Acrylamide Powder	500g
<u>786-508</u>	Acrylamide Solution, 40%	500ml
RC-024	Bis (N,N'-methylenebisacrylamide) Powder	50g
RC-025	Bis (N,N'-methylenebisacrylamide) Powder	250g
786-509	Bis (N,N'-methylenebisacrylamide) Solution, 2%	500ml
RC-017	Ammonium Persulfate Powder	100g
<u>786-510</u>	Ammonium Persulfate, 100mg tablets	100 tablets
DG092	SDS: Sodium dodecyl sulfate	100g
RC-101	<u>TEMED</u>	50ml
<u>786-029</u>	SDS-PAGE Running Buffer [10X]	1L
786-029G	SDS-PAGE Running Buffer [10X]	1gal
<u>786-025</u>	SDS-PAGE Sample Loading Buffer [2X]	2.5ml
<u>786-420</u>	Tris Glycine Native Gel Running Buffer	1L
<u>786-421</u>	Native Sample Loading Buffer [2X]	2.5ml
<u>786-472</u>	Discontinuous Buffer System (425ml Anode buffer + 500ml Cathode buffer)	1 kit
<u>786-475</u>	Tricine Sample Buffer [2X]	30ml
<u>786-479</u>	Tris-Tricine [10X] (1M Tris, 1M Tricine, pH8.3)	1L
<u>786-480</u>	Tris-Tricine-SDS [10X] (1M Tris, 1M Tricine, 1% SDS, pH8.3)	1L
<u>786-481</u>	Zymogram Development Buffer [10X] (0.5M Tris.HCL, 2M NaCl, 50mM CaCl ₂ , 0.2% Brij-35, pH 7.5)	125ml
<u>786-482</u>	Zymogram Renature Buffer [10X]	125ml
<u>786-483</u>	Zymogram Sample Buffer	30ml
<u>786-484</u>	IEF Anode Buffer [10X]	250ml
<u>786-485</u>	IEF Cathode Buffer (pH 3-10) [10X]	250ml
<u>786-486</u>	IEF Cathode Buffer (pH 3-7) [10X]	250ml
<u>786-531</u>	MES SDS running buffer [20X]	500ml
786-532	MOPS/ SDS Running Buffer [20X]	500ml

MASS SPECTROMETRY COMPATIBLE PROTEIN GEL STAINS

LabSafe GEL Blue■

Ultra-sensitive, fast, ready-to-use protein stain

LabSafe GEL Blue™ is an enhanced protein stain that is based on Coomassie dye that offers unsurpassed sensitivity and rapid band visualization. LabSafe GEL Blue[™] is supplied in a ready-to-use format. which is added directly to protein gels following electrophoresis, after a brief wash step.

LabSafe GEL Blue™ is a sensitive stain that is able to detect as little as 4ng protein. Protein bands are visible in as little as three minutes, with maximal staining achieved in an hour. The figure shows that LabSafe GEL Blue™ can detect protein levels as low as 4ng BSA. 60-1000ng BSA is detectable in 5-10 minutes and the low levels of BSA (4-8ng) become clearly visible when washed in water.

Supplied as a liter; sufficient reagent to stain up to 50 mini gels (8 x 10cm) or as a 1 gallon size for staining up to 200 mini gels.

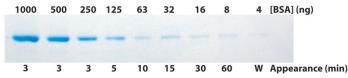


Figure 30: The sensitivity and speed of protein band visualization with LabSafe GEL Blue™. Serial dilutions (4ng-1mg) of bovine serum albumin (BSA) were reduced and loaded onto a SDS gel. Following electrophoresis, the gel was washed in deionized water to remove excess SDS. LabSafe GEL Blue™ was added and shaken for 1 hour. Protein bands (>250ng) appeared in 3 minutes and all bands >8ng were visible after 60 minutes. The gel was washed (W) in water to reveal the 4-8ng protein.

FEATURES

- Sensitive, 4-8ng BSA
- Saves time, band develops within three minutes
- Avoids the use of methanol, acetic acid, or other toxic agents

APPLICATIONS

- Staining of protein electrophoresis gels
- For native PAGE, SDS-PAGE, isoelectric focusing, or 2D gels

CITED REFERENCES

- Riascos, J. J. at al (2016) The seed biotinylated protein of soybean (Glycine max): A boilingresistant new allergen (Gly m 7) with the capacity to induce IgE-mediated allergic responses. J. Agric. Food Chem.DOI: 10.1021/acs.jafc.5b05873."
- Kanauiia, P.K. et al (2015) Proteomic analysis of Yersinia enterocolitica biovar 1A under iron-rich and iron-poor conditions indicate existence of efficiently regulated mechanisms of iron homeostasis J Proteomics, 124:39
- Keffer, C.R. et al (2015) Appl Environ Microbiol 81:3442
- Lara-Chavez, A. et al (2015) J. Exp. Bot. DOI: 10.1093/jxb/erv096
- Bhalla, A. et al (2014) BMC Biotechnol. 14:106
- Tsen, S.D. et al (2014) PLOS. DOI: 10.1371/journal.pone.0111673
- Lawrence, J. et al (2014) J. Neurosci, DOI: 10.1523/JNEUROSCI.0326-14.2014
- Khatibi, P. A. et al (2014) Biotechnol Biofuels. doi:10.1186/1754-6834-7-104
- McGraw, J. et al (2014) Protein Expres. Purif. 96:48

Cat. No.	Description	Size
<u>786-35</u>	<u>LabSafe GEL Blue</u> [™]	1L
786-35G	LabSafe GEL Blue [™]	1gal

FOCUS™ FASTsilver™

Mass spectrometry compatible silver stain for enhanced spot visualization

FOCUS™ FASTsilver™ produces crystal clear backgrounds and maximal peptide recovery needed for critical analysis by mass

For mass spectrometry analysis, complete proteolytic digestion and recovery of peptides is required for optimal analysis, however silver ions in traditional silver staining kits inhibit proteolytic digestion. In addition, glutaraldehyde, a common sensitizer in silver stains, modifies peptide lysine residues preventing complete digestion and recovery.

FOCUS™ FASTsilver™ produces high quality silver staining without the use of glutaraldehyde and is supplied with a highly efficient silver ion removal reagent, SilverOUT™. SilverOUT™ removes silver ions, which permits complete peptide digestion and extraction of peptides for maximal recovery.



Figure 31: A 2D electrophoresis gel stained with FOCUS™ FASTsilver™.

FEATURES

- Protease digestion uninhibited: Supplied with SilverOUT™ to completely remove silver ions that inhibit protease digestion by binding at the active sites of various proteases. This allows for optimal protease digestion
- · Complete peptide recovery: Glutaraldehyde-free silver stain that results in no lysine modification, protein cross linking or reduced peptide recovery. FOCUS™ FASTsilver™ allows enhanced protease digestion and efficient recovery of digested peptides
- Sensitivity: ~0.3ng protein and crystal clear background for maximum sensitivity
- Short protocol time: Protein can be detected in less than 90 minutes

APPLICATIONS

- · Staining of proteins in electrophoresis gels
- Fully compatible with mass spectroscopy analysis

- Rawal, R. et al (2016) Towards a Proteomic Catalogue and Differential Annotation of Salivary Gland Proteins in Blood Fed Malaria Vector Anopheles culicifacies by Mass Spectrometry PLOS doi.org/10.1371/journal.pone.0161870
- Yeh, F.L. et al (2016) Detection of Protein-Protein Interaction Within an RNA-Protein Complex 2. Via Unnatural-Amino-Acid-Mediated Photochemical Crosslinking. Methods Mol Biol.1421:175
- Aslam, M.K. et al (2015) Theriogenology doi:10.1016/j.theriogenology.2015.04.020
- Vijay, S. et al (2015) PLOS. DOI: 10.1371/journal.pone.0119666 Tham, J. M. et al (2015) Methods Mol. Biol. 1270:205
- Olson, K.E. et al (2014) PLOS. DOI: 10.1371/journal.pone.0086473
- Berg, M. et al (2014) PLOS. DOI: 10.1371/journal.pone.0089436 MacLeod, G. et al (2014) Reproduction. 147:1
- Kharenko, O.A. et al (2013) J. Biochem.154:383

Cat. No.	Description	Size
786-240T	<u>FOCUS</u> [™] <u>FASTsilver</u> Kit	5 mini Gels
786-240	FOCUS [™] FASTsilver Kit	25 mini Gels

Reversible Zinc Stain[™]

Stains hard to stain proteins, including glycoproteins & phosphoproteins

A single step stain for the rapid detection of proteins resolved by PAGE (native gels or SDS denatured gels). No destaining is necessary.

The stain is based on the interaction of zinc ions with polyacrylamide and proteins. The stain works by depositing a zinc metal precipitate in the gel which turns the gel opaque white, while the SDS coating on the proteins prevents the stains from binding to the proteins. A negative image of the gel is produced; clear protein bands are detected against a semi-opaque white polyacrylamide background. Protein bands are visualized in as little as 10-15 minutes.

The sensitivity of the *Reversible Zinc Stain*^{\mathbb{M}} is 0.1-0.5ng BSA protein and does not interfere with electroelution of proteins or alter their biological properties. Gels stained with the *Reversible Zinc Stain*^{\mathbb{M}} can be destained in 5 minutes before the transfer or electroelution of proteins.

This stain works with native, SDS denatured gels and gels containing glycine, tricine and a variety of primary amine containing buffers.

Reversible Zinc Stain[™] has a unique property that in some situations makes it far superior than the highly sensitive silver stains. Silver staining, although very sensitive, is known not to detect certain types of proteins, including glycoproteins. Reversible Zinc Stain[™], having comparable sensitivity, is able to stain glycoproteins. The figure shows the difference in glycoprotein staining between a silver stain and Reversible Zinc Stain[™].

FEATURES

- For proteins difficult to detect with silver stains, i.e. glycoproteins
- Suitable for staining of protein gels including native and SDS denatured gels and gels containing Glycine and Tricine
- Staining suitable for subsequent protein elution, blotting, sequencing and mass spectroscopy

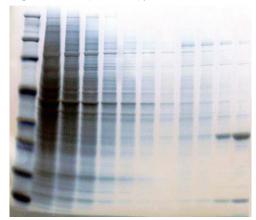


Figure 32: The use of Reversible Zinc Stain™ to follow glycoprotein extraction. The samples were prepared using the FOCUS™ Glycoprotein kit and the purified glycoproteins were easily visualized with the Reversible Zinc Stain™. Low levels of glycoproteins are notoriously difficult to stain with other stains.

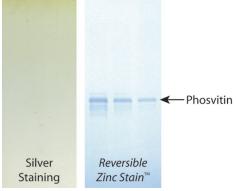


Figure 33: Phosvitin, a phosphoglycoprotein, was separated by SDS-PAGE and developed either by a silver staining method or the Reversible Zinc Stain $^{\mathbb{N}}$. Phosvitin was only visualized with the Reversible Zinc Stain $^{\mathbb{N}}$.

CITED REFERENCES

1. Becks, L. et al (2010) BMC Cancer. 10:540

Cat. No.	Description	Size
786-32ZN	Reversible Zinc Stain [™]	25 mini gels
786-32DSZN	Destaining Solution-Zinc [10X]	500ml

RUBEO™ Fluorescent Protein Stain

RUBEO™ is a fluorescent protein stain that is ideal for staining proteins in 1D and 2D electrophoresis gels. The fluorescent stain has a red emission at 605nm. The fluorescent stain has nanogram sensitivity and is compatible with mass spectroscopy, microsequencing and immunostaining. The stained gels can be visualized on a simple UV transilluminatoror the most complex laser scanners.

Available in 200ml and 1L size suitable for 4 or 20 mini gels.

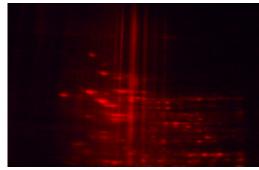


Figure 34: Polyacrylamide gel stained with RUBEO™.

FEATURES

- Fluorescent: Red emission at 605nm.
- · High Sensitivity: Easily detect ng levels of protein.
- Minimal protein protein variation: Allows quantitative comparison of proteins.
- Compatible: Ideal for subsequent analysis, including mass spectroscopy, microsequencing and immunostaining.
- Versatile: Stain native, denaturing, IEF and 2D gels.
- Suitability: Visualize with the simplest UV transilluminators to the complex laser scanners.

APPLICATIONS

- · For staining of protein electrophoresis gels
- · Mass spectrometry analysis

CITED REFERENCES

Harris, L.R. et al (2007) J. Proteome. Res. 6:1418

Cat. No.	Description	Size
<u>786-644</u>	<u>RUBEO</u> ™	200ml
786-645	RUBEO™	1L

Trypsin for Mass Spectrometry

Trypsin is a serine endopeptidase that specifically cleaves peptide bonds on the carboxy side of s-aminoethyl cysteine, arginine and lysine residues. Typically there is little or no cleavage at arginyl-proline and lysyl-proline bonds.

Trypsin undergoes autolysis, producing trypsin fragments that interfere with sequence analysis. G-Biosciences' mass spectrometry grade trypsin is a chemically modified trypsin that is enzymatically active and yet resistant to autolysis. Mass spectrometry grade trypsin is methylated, TPCK treated and quality tested for mass spectrometry.

Unlike other trypsin preparations, mass spectrometry grade trypsin is highly stable, maintaining its activity in severe denaturing buffers and as a result, is shipped without requiring dry ice and can be stored for a long period without any loss of activity.

We supply two sources of mass spectrometry grade trypsin, either bovine or porcine.

For mass spectrometry sequence analysis, mass spectrometry grade trypsin to protein ratio of 1:20 to 1:100 is recommended. For convenience, mass spectrometry grade trypsin is supplied in 20µg, 100µg and 200µg vials with a specific resuspension buffer.

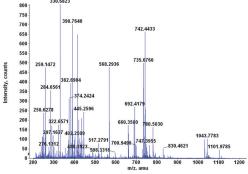


Figure 35: MALDI-TOF Mass Spectrum of casein digested with our mass spectrometry grade trypsin.

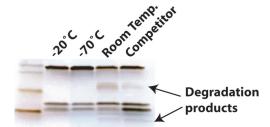


Figure 36: Mass spectrometry grade trypsin is highly stable. Stored at -20°C, -70°C and room temperature for six months and then resuspended and analyzed by SDS-PAGE and stained with FOCUS FastSilver™; For a comparison, a competitor's trypsin was resuspended according to the manufacturer's protocol and an equivalent amount was analyzed. Only our mass spectrometry grade trypsin stored at room temperature and the competitor's trypsin showed degradation products.

FEATURES

- Ultra pure porcine or bovine trypsin
- · Modified by methylation and TPCK treatment
- · Resistant to autolysis and degradation
- · For sequence analysis and mass spectrometry applications
- Stable at ambient temperature and suitable for long term storage
- Specific activity >10,000U/mg protein

CITED REFERENCES

- Schacherer, L. et al (2017) Quantification of intractable membrane proteins in genetically engineered crops by liquid chromatography coupled with tandem mass spectrometry. Anal. Methods DOI: 10.1039/C7AY00161D
- Polevoda, B. et al (2017) DNA Mutagenic Activity and Capacity for HIV-1 Restriction of the Cytidine Deaminase APOBEC3G Depends on Whether DNA or RNA Binds to Tyrosine 315. J Biol Chem. doi: 10.1074/jbc.M116.767889

More cited references available at www.gbiosciences.com

Cat. No.	Description	Size
<u>786-245</u>	Trypsin, Mass Spectrometry Grade (Porcine)	5 x 20µg
786-245B	Trypsin, Mass Spectrometry Grade (Bovine)	5 x 20µg
786-687B	Trypsin, Mass Spectrometry Grade (Bovine)	100µg
<u>786-687</u>	Trypsin, Mass Spectrometry Grade (Porcine)	100µg
<u>786-688</u>	Trypsin, Mass Spectrometry Grade (Porcine)	200µg
<u>786-690</u>	Trypsin, Mass Spectrometry Grade (Porcine)	5 x 100μg
786-693	Trypsin, Mass Spectrometry Grade (Porcine)	5 x 200μg

SEQUENCING GRADE PROTEASES

SG-Chymotrypsin[™]

A serine endopeptidase, which predominantly cleaves peptide bonds on the carboxy side of tyrosine, phenylalanine and tryptophan. In addition, chymotrypsin has a low catalytic activity against the carboxy side of leucine, methionine, alanine, aspartic and glutamic acids. It is therefore recommended to always use the shortest digestion time possible.

SG-Chymotrypsin™ is first treated with TLCK to inhibit trypsin that may be present and then subjected to an extensive purification process to remove contaminating protease and chymotryptic autolysis by-products. The highly purified enzyme is then chemically modified to increase its resistance to autolysis and stability.

Use at a ratio of 1:200 to 1:50, by weight, in a standard digestion buffer. Incubate at 25-30 °C for 1 to 10 hours, but can be extended to 24 hours.

Cat. No.	Description	Size
<u>786-13</u>	<u>SG-Chymotrypsin</u> [™]	2 x 25µg

SG-Lysine-C™

An endopeptidase, from Lysobacter enzymogenes, is a serine protease highly specific in cleaving peptide bonds at the carboxy side of lysine. Highly purified preparations of SG-Lysine- $\mathbb{C}^{\mathbb{N}}$ are chemically modified making the enzyme resistant to autolysis and stabilizing its enzymatic activity.

SG-Lysine- C^{∞} is supplied lyophilized in 5µg vials. The enzyme is typically reconstituted to a concentration of 0.25µg/ml. For fragmentation, the enzyme is added to the sample protein in a ratio of 1:100 to 1:20 (enzyme to protein, by weight) in a standard digestion buffer.

Cat. No.	Description	Size
786-14	SG-Lysine-C [™]	2 x 5µg

SG-Glutamic-C™

A serine endopeptidase, from *Staphylococcus aureus V8*, that is highly specific for the cleavage of peptide bonds at the carboxy side of either aspartic or glutamic acid, depending on the buffer used. In Tris-HCl buffer, in particular in the absence of phosphate ions, the enzyme is specific for the glutamyl site. Recommended buffers for fragmentation of proteins using this enzyme are 50mM Tris-HCl, pH 8.0 or bicarbonate buffer. Highly purified preparations of SG-Glutamic-C™ are chemically modified making the enzyme both resistant to autolysis and stabilizes its enzymatic activity.

SG-Glutamic- C^{∞} is supplied lyophilized in 10µg vials. The enzyme is typically reconstituted to a concentration of 0.5µg/ml and commonly used at a ratio of 1:100 to 1:20 (enzyme to protein, by weight) in a standard digestion buffer.

CITED REFERENCES

1. Bernardes, C.P. et al (2013) J. Proteomics. 80:250

Cat. No.	Description	Size
<u>786-15</u>	SG-Glutamic-C [™]	2 x 10µg

SG-Arginine-C[™]

An endopeptidase (Clostripain, from *Clostridium histolyticum*) specifically hydrolyzes the carboxy peptide bond of Arginine. SG-Arginine- $\mathbb{C}^{\mathbb{M}}$ has been modified chemically by a propriety process to render the enzyme resistant to autolysis and stabilize enzymatic activity. In addition, as a sulfhydryl enzyme, SG-Arginine- $\mathbb{C}^{\mathbb{M}}$ is susceptible to inactivation by oxidation and as a result requires reducing agents for protection. The enzyme also requires calcium ion for maximal activity. A special reconstitution buffer is supplied, which contains reducing agents and activators to maintain enzyme activity.

SG-Arginine-C[™] is supplied lyophilized in an activated form in 5 μ g vials and can be reconstituted to a concentration of 0.25 μ g/ml by addition of 20 μ l per vial of the supplied reaction buffer. For fragmentation the enzyme is added to the sample protein in a ratio of 1:100 to 1:20 (enzyme to protein, by weight).

CITED REFERENCES

 Wang, W. et al (2017) Abnormal levels of histone methylation in the retinas of diabetic rats are reversed by minocycline treatment. Sci Rep. doi:10.1038/srep45103

Cat. No.	Description	Size
<u>786-11</u>	<u>SG-Arginine-C</u> [™]	2 x 5µg

Immobilized Trypsin

Immobilized Trypsin is TPCK treated trypsin immobilized on 4% agarose that eliminates the contamination of protein digests by the trypsin. The immobilized trypsin is readily removed by separating the agarose from the digestion solution.

Trypsin is a serine endopeptidase that specifically cleaves peptide bonds on the carboxy side of s-aminoethyl cysteine, arginine and lysine residues and typically there is little or no cleavage at arginyl-proline and lysyl-proline bonds. The distribution of these residues in proteins allows trypsin digestion to produce peptides that are readily identified by mass spectrometry.

Native trypsin is prone to autolysis that results in pseudotrypsin, which exhibits a broader proteolytic specificity (a chymotrypsin like activity) and trypsin fragments that interfere with sequence analysis.

The trypsin is TPCK treated to inactive the interfering chymotrypsin activity and the resulting protein is affinity purified. Immobilzed Trypsin is supplied as a 50% slurry containing glycerol and sodium azide as a preservative.

FEATURES

- · Eliminate contamination with trypsin
- · Source: Bovine
- . Activity: ≥200 TAME units/ml resin
- Support: 4% Cross-linked Agarose

Cat. No.	Description	Size
786-792	Immobilized Trypsin	2ml Resin

SG-Carboxypeptidase B (Recombinant)™

SG-Carboxypeptidase B (Recombinant)™is the rat carboxypeptidase B expressed in E. coli. Carboxypeptidase B specifically hydrolyses basic amino acids including lysine, arginine and histidine from the C-terminal end of polypeptides.

FEATURES

- Animal free source of origin, therefore no any other contaminating proteases:
- No protease inhibitors are present during preparation of SG-Recombinant Carboxypeptidase B™
- High Purity: HPLC grade; single band on SDS-PAGE; no other contaminating proteases such as chymotrypsin and carboxypeptidase A. Less than 10ppm of recombinant trypsin.

APPLICATIONS

 SG-Carboxypeptidase B (Recombinant)[™] is used in sequencing protein and peptides.

Cat. No.	Description	Size
786-1249	SG-Carboxypeptidase B (Recombinant)™	0.1 mg
786-1250	SG-Carboxypeptidase B (Recombinant)™	1 mg

SG-Chymotrypsin (Human, Recombinant)™

SG-Chymotrypsin (Human, Recombinant)™ is recombinant human chymotrypsin expressed in E. coli and purified by HPLC method.

Chymotrypsin hydrolysis at the carboxyl side of aromatic amino acid residues including Tyrosine, phenylalanine and Tryptophan. Cleavage occurs at lower rate at Leucine and methionine residues.

FEATURES

- Animal free source of origin, therefore no any other contaminating proteases.
- · High purity:>95%, purified with HPLC

APPLICATIONS

 Chymotrypsin is used peptide mapping (mass spectrometry), fingerprinting and sequence analysis alone or along with other proteases.

Cat. No.	Description	Size
<u>786-1251</u>	SG-Chymotrypsin (Human, Recombinant)™	0.1 mg
786-1252	SG-Chymotrypsin (Human, Recombinant)™	1 mg

Trypsin (Human, Recombinant)

Trypsin is a serine protease that cleaves peptides on C-terminal end of lysine and arginine amino acid residues. The pH optimum of trypsin is pH 7.0-8.0. Trypsin is inhibited by serine protease inhibitors including TLCK (N-p-tosyl-L-lysine chloromethyl ketone), PMSF (phenylmethanesulfonyl fluoride), benzamidine, soybean trypsin inhibitor, and ovomucoid

Trypsin (Human, Recombinant) is genetically engineered human trypsin expressed in E. coli and purified by high pressure liquid chromatography. It has animal free source of origin, so is virus free and also it has no other contaminating proteases such as chymotrypsin and carboxypeptidase. No protease inhibitor such as PMSF involved in its preparation.

FEATURES

- Animal free source of origin: Recombinant human trypsin expressed in E. coli. Free from contaminating proteases such as chymotrypsin and carboxypeptidase A and viruses.
- High purity: ≥ 95%; purified by high pressure liquid chromatography

APPLICATIONS

- Trypsin (Human, Recombinant) can be used to make celldissociation reagents
- It can be used for digestion of peptide and proteins for sequencing.

Cat. No.	Description	Size
<u>786-1253</u>	Trypsin (Human, Recombinant)	1 mg
<u>786-1254</u>	Trypsin (Human, Recombinant)	5 mg
786-1255	Trypsin (Human, Recombinant)	50 mg

IN GEL DIGESTION

InGel[™] Silver

In gel digestion of proteins in silver stained gels

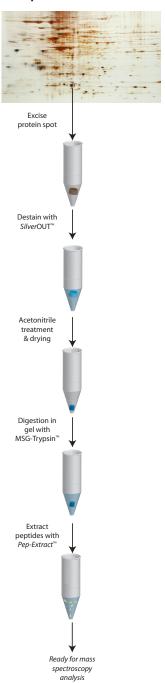


Figure 37: InGel[™] silver scheme.

InGel™ Silver provides a complete set of reagents for the in gel tryptic digestion and extraction of peptides for mass spectrometry (MALDI and LC MS/MS). The kit is specifically designed for use with silver stained protein spots/bands.

The protein spots are first excised from the silver stained gel and transferred to a proteomic grade tube. Silver stained gel pieces are washed with SilverOUT™ to remove inhibitory silver ions. The protein is then alkylated and reduced within the gel piece using the supplied aliquots of DTT and iodoacetamide. The proteins are then digested within the gel using our Mass Spectrometry Grade Trypsin and proprietary Digestion Buffer.

The digested peptides are extracted with Pep-Extract $^{\mathbb{M}}$, a high diffusion peptide extraction buffer. The extracted peptides are suitable for mass spectrometry analysis without any subsequent treatments or cleaning procedures.

InGel™ Silver is supplied with:

- SilverOUT™: For removal of silver ions
- OneQuant™ DTT: Reducing agent in single use aliquots to prevent contamination
- OneQuant™ lodoacetamide: Alkylating agent in single use aliquots to prevent contamination
- Trypsin, Mass Spectrometry Grade: Highly pure, autolysis resistant trypsin
- . Trypsin Digestion Buffer: For optimal trypsin activity
- Pep-Extract[™]: For high level peptide extraction

FEATURES

- For the in-gel tryptic digestion of proteins
- · Compatible with silver stained proteins
- · Supplied with Mass spectrometry grade trypsin
- Supplied with destaining, reducing, alklating and peptide extraction reagents

APPLICATIONS

For MALDI peptide mass mapping and for LC MS/MS

CITED REFERENCES

- Kanaujia, P.K. et al (2015) Proteomic analysis of Yersinia enterocolitica biovar 1A under iron-rich and iron-poor conditions indicate existence of efficiently regulated mechanisms of iron homeostasis J Proteomics. 124:39
- 2. Gahlaut, A. and Dabur, R. (2014) Int. J. Pharm. Pharm. Sci. 6:784
- Sharma, R. et al (2013) J. Mol. Catal. B-Enzym. 91:8
- 4. Hassan, B.H. and Cronan, J.E. (2011) JBC. 286:8263

Cat. No.	Description	Size
<u>786-241</u>	<u>InGel[™] Silver</u>	100 preps

InGel[™] Blue

In gel digestion of proteins in Coomassie and fluorescent stained gels

Provides a complete set of reagents for the ingel tryptic digestion and extraction of peptides for mass spectrometry (MALDI and LC MS/MS). The kit is specifically designed for use with Coomassie or fluorescent stained protein spots/bands.

The protein spots are first excised from the Coomassie or fluorescent stained gel. Stained gel pieces are washed with BlueOUT™ to remove inhibitory stains. The protein is then alkylated and reduced within the gel piece using the supplied aliquots of DTT and iodoacetamide. The proteins are digested within the gel using our Mass Spectrometry Grade Trypsin and proprietary Digestion Buffer.

The digested peptides are extracted with Pep-Extract™, a high diffusion peptide extraction buffer. The extracted peptides are suitable for mass spectrometry analysis without any subsequent treatments or cleaning procedures.

InGel™ Blue is supplied with:

- BlueOUT™: For removal of Coomassie or fluroescent stains
- OneQuant™ DTT: Reducing agent in single use aliquots to prevent contamination
- OneQuant™ Iodoacetamide: Alkylating agent in single use aliquots to prevent contamination
- Trypsin, Mass Spectrometry Grade
- Trypsin Digestion Buffer: For optimal trypsin activity
- Pep-Extract[™]: For high level peptide extraction

FEATURES

- · For the in-gel tryptic digestion of proteins
- · Compatible with Coomassie and fluorescent stained proteins
- Supplied with Mass spectrometry grade trypsin
- Supplied with destaining, reducing, alklating and peptide extraction reagents

APPLICATIONS

• For MALDI peptide mass mapping and LC MS/MS

CITED REFERENCES

 Priyanka, A. et al (2016) Crystal structure of the N-terminal domain of human SIRT7 reveals a three-helical domain architecture. Proteins. DOI: 10.1002/prot.25085"

Cat. No.	Description	Size
786-681	<u>InGel[™] Blue</u>	100 preps

InGel[™] Array

High throughput in gel digestion of protein spots

96-well format kit to process larger numbers of protein spots concurrently and is compatible with spot-picking instruments.

The protein spots are first excised from the silver stained gel and transferred to a proteomic grade titer plate. Silver stained gel pieces are washed with SilverOUT™ to remove inhibitory silver ions. The proteins are then digested within the gel using a Mass Spectrometry Grade Trypsin and supplied Digestion Buffer.

The digested peptides are extracted with Pep-Extract $^{\mathbb{M}}$, a high diffusion peptide extraction buffer. The extracted peptides are suitable for mass spectrometry analysis without any subsequent treatments or cleaning procedures.

InGel[™] Array is supplied with:

- SilverOUT™: For removal of silver ions
- Trypsin Digestion Buffer: For optimal trypsin activity
- Pep-Extract[™]: For high level peptide extraction
- InGel[™] Array titer plates and caps

Mass Spectrometry Grade Trypsin is available separately.

Cat. No.	Description	Size
786-241A	InGel [™] Array	500 preps

ACCESSORIES

Silver**0**UT[™]

A highly efficient destaining solution to remove interfering silver ions from gel pieces. Gel spots can be destained in a little as 10 minutes. Fully compatible with downstream processes, including mass spectrometry.

BlueOUT[™]

Blue-OUT[™] is a unique product that washes the interfering Coomassie and fluorescent protein stains from gel pieces prior to their treatment for protein sequencing or mass spectrometry.

Pep-Extract[™]

Following in gel digestion of proteins with proteases, the addition of Pep-Extract™ rapidly elutes digested peptides in under 20 minutes. The eluted peptides can be used directly in mass spectrometry analysis, without any additional clean up.

Trypsin Digestion Buffer

The Trypsin Digestion Mix provides optimal buffered conditions for in gel trypsin digestion of proteins.

Proteomic Grade Water

Ultra pure proteomic grade water that is fully compatible with 2D electrophoresis and mass spectrometry analysis.

ProteinOUT[™]

Mass spectrometry compatible washing solution

A unique washing solution that cleans plastic and glassware used in the proteolytic digestion and peptide isolation for mass spectrometry analysis. The rinsing of plastic and glassware with ProteinOUT™ will remove interfering chemicals and peptides that can leach from the tube walls and interfere or suppress the mass spectrometry signal. In addition, rinsing with ProteinOUT™ removes inadvertent contamination with dust and keratin. A simple rinse will prevent the need for repeat experiments, saving both time and money. A more cost effective alternative to siliconized tubes. For the rinsing of ~200 1.5-2ml centrifuge tubes.

Proteomic Grade Microfuge Tubes

1.5ml centrifuge tubes that are certified protein/dust free. Suitable for proteomic analysis and research.

CITED REFERENCES

1. Petrica, L. et al (2015) Int. J. Clin. Exp. Med. 8(2):2516

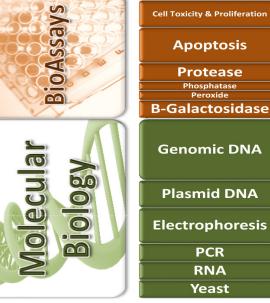
Cat. No.	Description	Size
786-244	<u>SilverOUT</u> [™] _	100 preps
<u>786-683</u>	BlueOUT [™]	100 preps
786-243	Pep-Extract [™]	500 preps
<u>786-242</u>	Trypsin Digestion Buffer	100 preps
786-229	Proteomic Grade Water	1L
786-680	<u>ProteinOUT</u> [™]	50ml
786-300	Proteomic Grade Microfuge tubes	100 tubes

G-Biosciences Product Line Overview

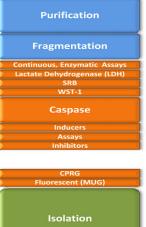








Estimation Extraction & Lysis Fractionation & Enrichment **Isolation Sample Preparation Electrophoresis Western Blotting Detection Mass Spectrometry** Assays (ELISA) **Affinity Resins Purification Activated Resins Antibody Purification** Labeling Crosslinkers Reducing Agents Alkylating Agents Protein Cleavage Modification Production **Antibody** Purification Fragmentation SAM Methyltransferase **Cell Toxicity & Proliferation**



CPRG
Fluorescent (MUG)
Fluorescent (WOG)
Isolation
Isolation
Colony Screening
Transformation
Apparatus
Loading Dyes
DNA Ladders
Gel Extraction
Tag
dNTPs
Extraction
RNase Decontamination
Transformation

CB-X	
Non Interfering	
SPN	
RED 660	
dotMETRIC	
BCA	
СВ	l .
Sample Grinding	
	Mild Denaturin
Lysis Buffers	Strong Chaotrop
	Specialized
12 Fractionation Kits	
Dialysis (Micro)	
Concentration	
	Desalting
Contamination Removal	Detergent Remo
	General Cocktai
Protease Inhibitors	Species Specific
Trottease ministrations	Individual Inhibit
Detergents	marriada minor
Chaotropes	
citaderopes	2D Specific Kits
1D & 2D Reagents	Buffers & Reager
	Coomassie
Gel Stains	Silver
Gerstains	Reversible
4.11	Reversible
1 Hour System	Non-Animal
DI 11 A	
Blocking Agents	Animal
0 1 0 111 11	Non-Protein
Secondary Antibodies	
Chemiluminescence Detection	
Trypsin, Mass Spec Grade	
InGel Kits	
Coated Plates	
	Non-Animal
Blocking Agents	Animal
	Non-Protein
Secondary Antibodies	
Detection Reagents	
	Nickel resin
6X His Tag	Cobalt resin
ox nis rag	Copper resin
	Zinc Resin
GST Tag	Glutathione Res
Biotin Tag	Streptavidin Res
CBP Tag	Calmodulin Resi
Sulfhydryl reactive	-
Amine reactive	
Carboxyl reactive	
Drug/ Steroid reactive	
Protein A or G	
Pearl Resin	
Biotin	
Fluorescent Dye	
Enzyme (HRP/AP)	

Carrier Proteins
Peptide Coupling
Protein A or G Resin
Activated Resins
Pearl Resin
Thiophilic Resin
Ficin
Pepsin
Papain

Assays	
Substrates	
Inhibitors	

	Tissue
	Blood
	Plant
	Yeast
E	Bacteria
	Fungi
M	ouse Tail

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