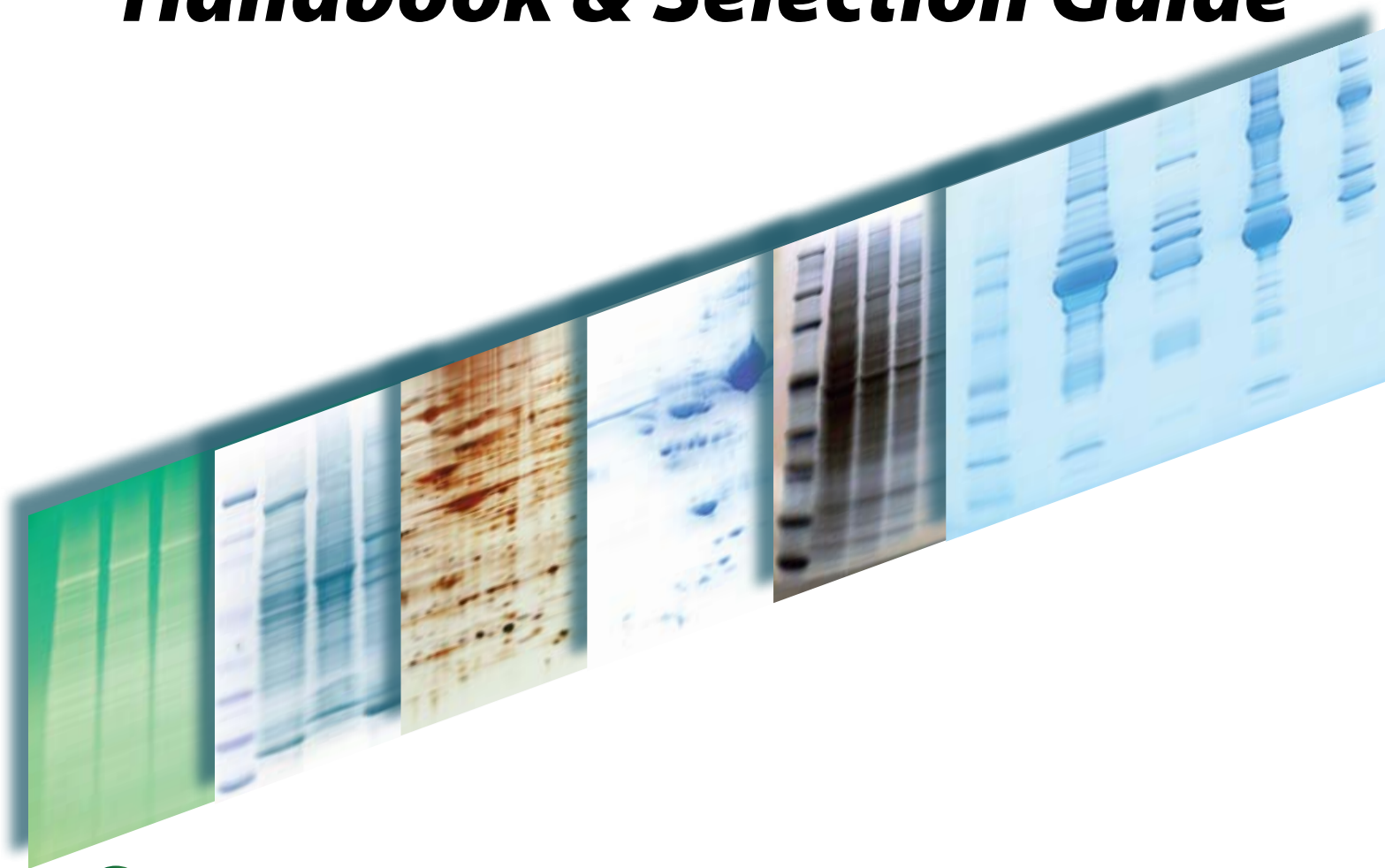


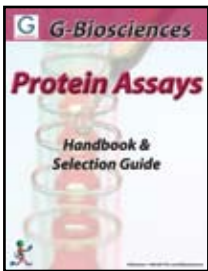


G-Biosciences

Protein Electrophoresis

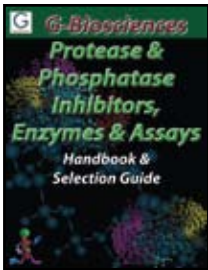
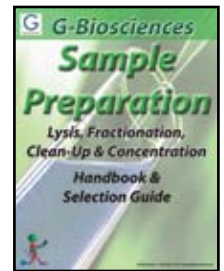
Handbook & Selection Guide





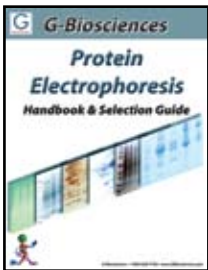
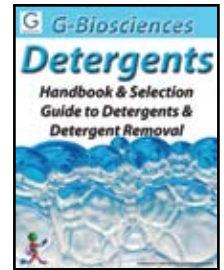
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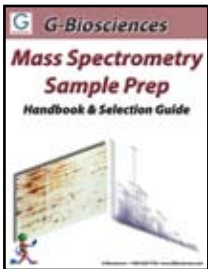
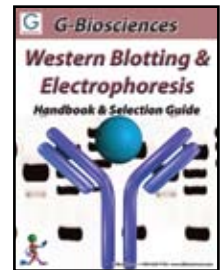
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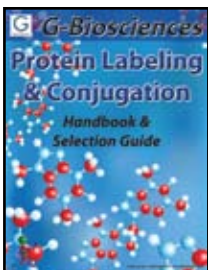
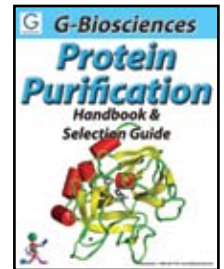
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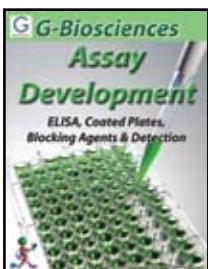
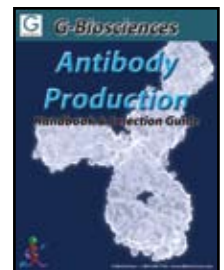
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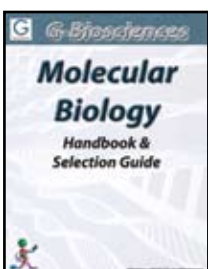
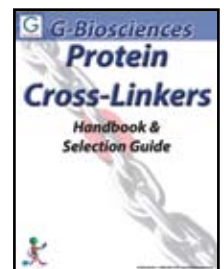
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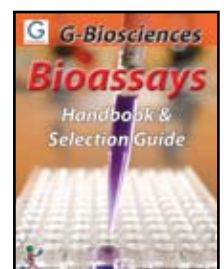
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Introduction

Protein electrophoresis is a routinely used technique in proteomic research that separates proteins based on their physical properties, including their molecular weight and their native charge (isoelectric point (pI)). The standard matrix used for protein separation is polyacrylamide in a process commonly known as PAGE (polyacrylamide gel electrophoresis).

Protein electrophoresis is a relatively simple, rapid and highly sensitive tool to study the properties of proteins. It is the principle tool in analytical chemistry, biochemistry, and molecular biology. The separation of proteins by electrophoresis is based on the fact that charged molecules will migrate through a matrix upon application of an electrical field.

The chemical agents used to form polyacrylamide are monomeric acrylamide and N, N'-methylene-bis-acrylamide (bis-acrylamide). The most popular method for polymerizing acrylamide and bis-acrylamide is using TEMED (tetramethylethylenediamine) and ammonium persulfate.

The size of pores in the polyacrylamide gel matrix is determined by the amount of total acrylamide used per unit volume and relative percentage of bis-acrylamide used. The effective range of polyacrylamide gel is between 3-30%.

Several different types of PAGE are used as an analytical or purification tool for proteins.

Non-Denaturing PAGE (Native PAGE): Separates proteins based on their native charge and mass.

SDS-PAGE: The most commonly used PAGE technique that separates proteins by their mass.

2D PAGE (Two dimensional PAGE): Combines two separations to first separate proteins by their isoelectric point and then by mass.

Two fundamentally different types of gel system exist, non-dissociating (non-denaturing) and dissociating (denaturing). Non-dissociating (non-denaturing) system is designed to separate native protein under conditions that preserve protein function and activity. In contrast, a dissociating system is designed to denature protein into their constituent's polypeptides and hence examines the polypeptide composition of samples.

Sodium dodecyl sulfate (SDS) is commonly used for denaturing proteins into their constituents and the method is known as sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (SDS-PAGE). Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis is the most commonly used system and this separates proteins strictly by their size.

SDS-PAGE uses two types of buffer systems: the continuous buffer system and the discontinuous buffer system. In the continuous buffer system the pH of the gel matrix remains constant throughout the separation. In contrast, the discontinuous buffer system consists of a narrow layer of stacking gel (of large pore size and acidic pH) above the main separating or resolving gel matrix of alkaline pH (pH 8.8). The stacking gel concentrates the protein sample before entering the separating gel and hence enhancing resolution. SDS-PAGE with a discontinuous buffer system is the most popular electrophoresis technique used to analyze polypeptides.

In SDS-PAGE, the protein mixture is denatured by heating at 100°C in the presence of excess SDS and a reducing reagent is employed to break disulfide bonds. Under these conditions, all reduced polypeptide bind the same amount of SDS on a weight basis (1.4g SDS/g polypeptide) independent of the amino acid composition and sequence of the protein. The SDS-protein complex forms a rod with its length proportional to the molecular weight of the protein. All proteins are now negatively charged with similar charge density and thus can be separated on the basis of their size only.

SDS-PAGE is used mainly for the following purpose:

1. Estimation of protein size.
2. Determination of protein subunits or aggregation structures.
3. Estimation of protein purity.
4. Protein quantitation.
5. Monitoring protein integrity.
6. Comparison of the polypeptide composition of different samples.
7. Analysis of the number and size of polypeptide subunits.
8. Post-electrophoresis applications, such as Western blotting.

2D Electrophoresis

Two-dimensional gel electrophoresis is a powerful tool for analyzing proteins and peptides based on their charge and mass. Unfortunately, 2D electrophoresis often leads to frustration due to issues with achieving good resolution and reproducibility. The basic principle of 2D electrophoresis is to use two separating techniques:

1. **Isoelectric Focusing (IEF):** Proteins or peptides are separated in an immobilized pH gradient based on their isoelectric point
2. **SDS-PAGE:** The IEF strip containing the separated proteins is then layed on a SDS-PAGE gel to further separate the proteins by mass.

G-Biosciences has a large range of products known as our FOCUS™ line of products. These products are designed to be fully compatible with 2D electrophoresis and include products for sample preparation, sample clean-up and the actual electrophoresis of the proteins or peptides.

SDS PAGE Gel Recipes

G-Biosciences provides high quality reagents for the preparation of homemade gels, including acrylamide, bis-acrylamide, TEMED, APS and buffers. The table below provides a recipe guide for the preparation of SDS-PAGE gels.

Percent Acrylamide Gel	Stacking Gel (6%)	Separating/ Resolving Gel				
		5%	7.5%	10%	12.5%	15%
Distilled Water (ml)	11.6	19.3	17.3	15.3	13.3	11.3
40% Acrylamide ¹ (ml) (Cat. # 786-502)	3	4	6	8	10	12
1.5M Tris, pH8.8 (ml)	-	8				
0.5M Tris, pH6.8 (ml)	5	-				
10% SDS (µl) (Cat. # R014)	200	320				
10% APS (µl) (Cat. # 786-510)	200	320				
TEMED (µl) (Cat. # RC-101)	20	32				

¹ 40% Solution, 38.96% solution containing acrylamide (40%) and bisacrylamide (1.04%) for cross-linker ratio of 37.5:1

Acrylamide/ Bisacrylamide

Acrylamide (Electrophoresis grade) is supplied as a powder or a 40% solution in ultrapure water.

Bisacrylamide (Bis (N,N'-methylenebisacrylamide)) (Electrophoresis grade) is supplied as a powder or a 2% solution in ultrapure water.

Acrylamide/Bisacrylamide Solutions are available at the most common ratios (37.5:1 or 29:1) for use in protein and nucleic acid electrophoresis. The concentration is based on the total weight of both the acrylamide and bisacrylamide. Supplied as 40% solutions Solutions prepared from electrophoresis grade acrylamide and bisacrylamide in ultra-pure water.

Acrylamide/Bisacrylamide Powders Ready to reconstitute dry powder blends are accurately pre-blended to produce a 40% (w/v) stock solution for use in protein and nucleic acid electrophoresis. The concentration is based on the total weight of both the acrylamide and bis-acrylamide. Available at the most common ratios (37.5:1 or 29:1). Eliminates the need to weigh toxic acrylamide and bisacrylamide.

Cat. #	Description	Size
RC-001	Acrylamide Powder	100g
786-508	Acrylamide Solution, 40%	500ml
RC-024	Bis (N,N'-methylenebisacrylamide) Powder	50g
786-509	Bis (N,N'-methylenebisacrylamide) Solution, 2%	500ml
786-502	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
786-503	Acrylamide/ Bisacrylamide (37.5:1); Premixed powder	40g
786-504	Acrylamide/ Bisacrylamide (37.5:1); Premixed powder	200g
786-505	Acrylamide/ Bisacrylamide (29:1); 40% Solution 40% solution containing acrylamide (38.67%) and bisacrylamide (1.33%) for cross-linker ratio of 29:1	500ml
786-506	Acrylamide/ Bisacrylamide (29:1); Premixed powder	40g
786-507	Acrylamide/ Bisacrylamide (29:1); Premixed powder	200g

SDS (Sodium Dodecyl Sulfate)

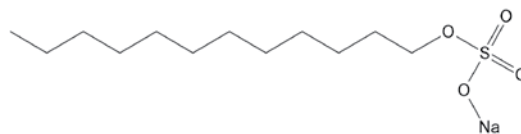


Figure 1: Structure of SDS.

Type: Anionic detergent

Mol. Formula: C₁₂H₂₅NaO₄S

Mol Weight: 288.38

Form: White to off white powder, 10% or 20% solution

Purity: >99%

Solubility: Water

Critical micelle concentration (CMC): 7-10mM (25°C)

Aggregation number: 62

Cloud point: >100°C

Average micellar weight: 18,000

Application: Capable of almost complete disruption of cellular structures and denaturation. Used for solubilization of a wide variety of proteins, including membrane proteins, for electrophoretic separation. Detergent molecules tightly bind with the protein molecules masking their native charge and rendering the protein molecules with an overall negative charge.

Cat. #	Description	Size
DG092	SDS	100g
DG093	SDS	500g
R014	SDS, 10% Solution	100ml
786-016	SDS, 20% Solution	500ml
786-017	SDS, 20% Solution	1L

Ammonium Persulfate (APS)

The catalyst for the polymerization of polyacrylamide gels. APS is available as a ready to use tablets or as a powder. For the tablets, simply add 1 tablet to 1.5ml ultrapure water for a 10% solution.

FEATURES

- Available as tablets or powder
- Synonym: Ammonium peroxodisulfate
- CAS#: 7727-54-0
- Molecular Formula: H₈N₂O₈S₂
- Molecular Weight: 228.18

Cat. #	Description	Size
RC-017	Ammonium Persulfate Powder	100g
786-510	Ammonium Persulfate, 150mg tablets	100 tablets

TEMED

Polymerization reagent of polyacrylamide gels.

FEATURES

- Purity >99.9%
- Synonym: N,N,N',N'-Tetramethylethylenediamine, 1,2-Bis(dimethylamino)ethane, TMEDA
- CAS#: 110-18-9
- Molecular Formula: C₆H₁₆N₂
- Molecular Weight: 116.24

Cat. #	Description	Size
RC-101	TEMED	50ml
RC-102	TEMED	100ml

PROTEIN MARKERS

PAGEmark™ Protein Ladder

Our prestained PAGEmark™ Markers are ~7 to >210kDa* ladder of eight proteins. PAGEmark™ proteins are covalently coupled to a blue dye and PAGEmark™ Tri-color proteins consist of six coupled to a blue dye, one to an orange dye and one to a red dye for easy reference. The PAGEmark™ protein ladders are available in two formats; a ready-to-use liquid format suitable for 100 x 10µl loads and a OneQuant™ format. The OneQuant™ format consists of 40 pre-aliquoted, lyophilized, ready-to-use markers, simply add 10µl water and load. The OneQuant™ PAGEmark™ Isingle use format prevents contamination from repeated withdrawals from the same tube.

The PAGEmark™ markers do not require boiling or denaturing; simply load on gel. PAGEmark™ are readily transferred to Western blot membranes (PVDF and nitrocellulose).

*Please visit www.GBiosciences.com for a Certificate of Analysis of precise, lot-specific molecular weights.

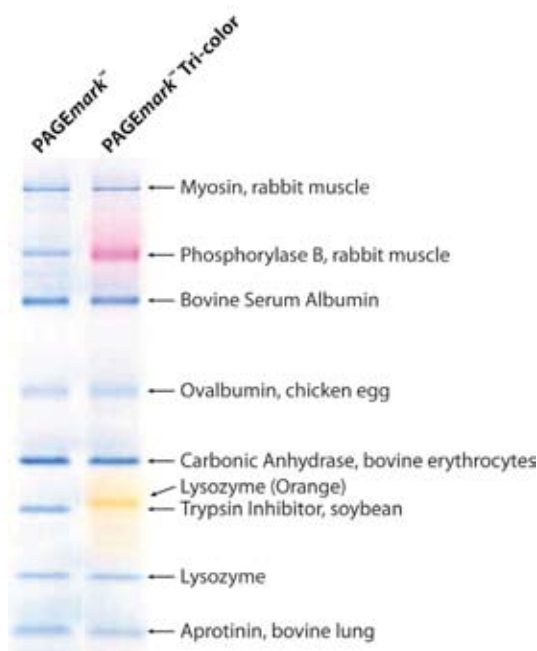


Figure 2: PAGEmark™ and PAGEmark™ Tri-color comparison.

FEATURES

- Protein markers for protein electrophoresis
- Wide range protein markers (~7 to >210kDa)
- Ready-to-use
- Single-use format available
- Blue or three colored prestained markers

Cat. #	Description	Size
786-412	PAGEmark™ Tri-color	100 loads
786-415	PAGEmark™	100 loads
786-416	OneQuant™ PAGEmark™	40 loads
786-417	OneQuant™ PAGEmark™ Tri-color	40 loads

ELECTROPHORESIS BUFFERS

For SDS-PAGE

SDS polyacrylamide gel electrophoresis is the most common protein electrophoresis that separates predominantly by protein mass.

Available buffers are:

- **SDS-PAGE Sample Loading Buffer [2X]:**
0.2M Tris, 10% Glycerol, 4% SDS, 0.01% Bromophenol Blue
- **SDS PAGE Running Buffer [10X]:**
0.24M Tris, 1.92M Glycine, 1% SDS

Cat. #	Description	Size
786-025	SDS-PAGE Sample Loading Buffer [2X]	2.5ml
786-029	SDS-PAGE Running Buffer [10X]	1L
786-029G	SDS-PAGE Running Buffer [10X]	1gal

For Native Gel Electrophoresis

Native gel electrophoresis separates proteins based on their native charge and mass.

Available buffers are:

- **Native Sample Loading Buffer [2X]:**
0.2M Tris, 10% Glycerol, 0.01% Bromophenol Blue
- **Tris Glycine Native Gel Running Buffer [10X]:**
0.24M Tris, 1.92M Glycine,

Cat. #	Description	Size
786-421	Native Sample Loading Buffer [2X]	2.5ml
786-420	Tris Glycine Native Gel Running Buffer [10X]	1L

For Tris Tricine Gel Electrophoresis

Tris Tricine Gel Electrophoresis is routinely used for the separation of small proteins and peptides with a molecular weight of <10kDa.

Available buffers are:

- **Tricine Sample Buffer [2X]:** 0.2M Tris, 2% SDS, 40% Glycerol, 0.04% Coomassie Blue, pH 6.8
- **Tris-Tricine [10X]:** 1M Tris, 1M Tricine, pH 8.3
- **Tris-Tricine-SDS [10X]:** 1M Tris, 1M Tricine, 1% SDS, pH 8.3

Cat. #	Description	Size
786-475	Tricine Sample Buffer [2X]	30ml
786-479	Tris-Tricine [10X]	1L
786-480	Tris-Tricine-SDS [10X]	1L

For Bis Tris Gel Electrophoresis

Bis Tris gels are polyacrylamide gels designed to give optimal separation of small- to medium-sized proteins under denaturing conditions. The gels can be run using either MES SDS running buffer or MOPS/SDS running buffer to obtain different separation ranges.

Available buffers are:

- **MES SDS Running Buffer [10X]:** 0.5M MES, 0.5M Tris, 1% SDS, 8mM EDTA, pH 7.25
- **MOPS SDS Running Buffer [20X]:** 1M MOPS, 1M Tris, 20.5mM EDTA, 2% SDS,

Cat. #	Description	Size
786-531	MES SDS running buffer [10X]	500ml
786-532	MOPS SDS Running Buffer [20X]	500ml

For Isoelectric Focusing (IEF)

Isoelectric focusing separated proteins based on their charge. The technique can be used on its own, but is more routinely used as the first dimension of 2D electrophoresis. Available buffers are:

- **IEF Anode Buffer [10X]:** 70mM Phosphoric Acid
- **IEF Cathode Buffer (pH3-10) [10X]:** 0.2M Lysine, 0.2M Arginine
- **IEF Cathode Buffer (pH3-7) [10X]:** 0.4M Lysine

Cat. #	Description	Size
786-484	IEF Anode Buffer [10X]	250ml
786-485	IEF Cathode Buffer (pH3-10) [10X]	250ml
786-486	IEF Cathode Buffer (pH3-7) [10X]	250ml

For Zymography

Zymography is an electrophoretic technique, based on SDS-PAGE, that includes an enzyme substrate copolymerized with the polyacrylamide gel. Samples are prepared in Zymogram Sample Buffer without boiling to preserve the structure and activity of the enzyme. Following electrophoresis, the SDS is removed from the gel by washing in Zymogram Renature Buffer that contains a non-ionic detergent. The gels are then equilibrated in Zymogram Developing Buffer, which contains the divalent metal cation required for enzymatic activity. The zymogram is subsequently stained (commonly with Amido Black or Coomassie Brilliant Blue), and areas of digestion appear as clear bands against a darkly stained background where the substrate has been degraded by the enzyme.

- **Zymogram Sample Buffer [2X]:** 62.5mM Tris, 4% SDS, 25% Glycerol, 0.01% Bromophenol Blue, pH 6.8
- **Zymogram Renature Buffer [10X]:** 25% Triton® X-100
- **Zymogram Development Buffer [10X]:** 0.5M Tris, 2M NaCl, 50mM CaCl₂, 0.2% Brij® 35, pH 7.5

Cat. #	Description	Size
786-483	Zymogram Sample Buffer [2X]	30ml
786-482	Zymogram Renature Buffer [10X]	125ml
786-481	Zymogram Development Buffer [10X]	125ml

Discontinuous Buffer System

For increased resolution and concentration of the protein sample band. Supplied as a two buffer system consisting of 425ml Anode Buffer and 500ml Cathode Buffer.

- **Anode Buffer [2X]:** 70.6mM Tris, 47.1mM CAPS, pH 9.6
- **Cathode Buffer [10X]:** 260mM Tris, 40mM CAPS, 0.1% SDS, pH 9.6

Cat. #	Description	Size
786-472	Discontinuous Buffer System	1 kit

PROTEIN REDUCTION & ALKYLATION

Protein-S-S-Reductant™

A water soluble, odorless, non-toxic and stable protein reductant. Protein-S-S-Reductant™ uses TCEP (Tris [2-carboxyethyl] phosphine), a popular alternative to β-mercaptoethanol and DTT (dithiothreitol). TCEP improves stability, increases effectiveness, and reduces proteins over a wider range of pH, including lower acidic pHs.

Protein-S-S-Reductant™ completely reduces stable disulfide bonds in less than 5 minutes at room temperature and is compatible with the protein alkylation reactions.

Protein-S-S-Reductant™ is a ready-to-use solution that is at neutral pH and stabilized for long-term storage.

Simply supplement Protein-S-S-Reductant™ in place of DTT or β-mercaptoethanol and boil the sample. TCEP powder is also available.

FEATURES

- Ready-to-use solution, odorless, stable and non-toxic
- Neutral protein reduction solution
- Compatible with the alkylation reaction
- Works over a wide range of pH, including lower acidic pHs

APPLICATIONS

- Reduction of protein disulfide bonds
- Reduction for protein electrophoresis and other applications

Cat. #	Description	Size
786-25PR	Protein-S-S-Reductant™	200 Preps
786-030	TCEP	1g

Dithiothreitol (DTT)

A common reducing agent used for the cleavage of disulfide bonds. DTT is supplied in bulk 5gm quantities.

OneQuant™ DTT are single aliquots of DTT that eliminate the need for weighing; preventing loss of reagent and saving time. Add 90μl water to a single tube to generate a 0.5M DTT solution. Supplied with 40 individual tubes.

Cat. #	Description	Size
BC99	DTT	5g
786-077	OneQuant™ DTT [0.5M]	40 vials

β-Mercaptoethanol

A popular reducing agent, is offered in 100ml bottles.

Cat. #	Description	Size
BC98	β-mercaptoethanol	100ml

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. #	Description	Size
786-232	FOCUS™ Protein Alkylation Kit	100 preps

Electrophoresis Separation

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

Cat. #	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. #	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. #	Description	Size
786-227	FOCUS™ DTT [2M]	5 x 1ml

Iodoacetamide

Iodoacetamide is supplied in 5 gram bulk quantities or in the OneQuant™ format. Add 150µl water to a single OneQuant™ vial to generate a 0.5M iodoacetamide solution.

Cat. #	Description	Size
786-228	Iodoacetamide	5g
786-078	OneQuant™ Iodoacetamide	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. #	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.

Cat. #	Description	Size
786-224	Equilibration Buffer for IPG Strips	For 50ml

Proteomic Grade Water

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

Cat.#	Description	Size
786-229	Proteomic Grade Water	1L

Proteomic Grade Tubes

Specifically prepared and certified protein and dust free.

Cat.#	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 (< 50µS). 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.

Cat.#	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 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

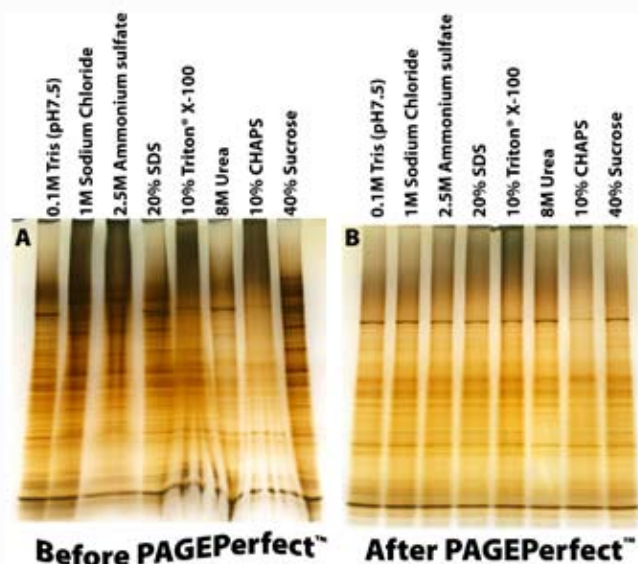


Figure 12: Analysis of mouse liver lysate before and after treatment with PAGE-Perfect™. A. 10µg mouse liver lysates, in the presence of various interfering agents, were loaded onto a SDS polyacrylamide gel. B. 10µg mouse liver lysates, in the presence of various interfering agents, were treated with PAGE-Perfect™ and then loaded onto a SDS polyacrylamide gel. Both gels were stained with FASTsilver™ protein.

Cat. #	Description	Size
786-123	PAGE-Perfect™	50 preps
786-123T	PAGE-Perfect™	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 13: 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

- Jang, S. et al (2007) *JBC*. 202: 929
 Laughton, J. et al (2006) *Microbiology*. 152:1155
 Devillard, E et al (2004) *J. Bacteriology*. 189: 136
 Rincon, M. et al (2004) *J. Bacteriology*. 186: 2576
 Jang, S. and Imlay, J. A. (2004) *Cancer Research*. 64: 9018

Cat. #	Description	Size
786-124	Perfect-FOCUS™	50 preps
786-124T	Perfect-FOCUS™	6 preps

COOMASSIE 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 3: 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

Stie, J., et al (2007) *J Leukoc. Biol.* 82:161
Taylor, R.M., et al (2006) *J Biol. Chem.* 281:37045

Cat. #	Description	Size
786-35	LabSafe GEL Blue™	1L
786-35G	LabSafe GEL Blue™	1gal

RAPIDstain™

An ultra sensitive and ready-to-use protein stain based on Coomassie dye. No mixing or preparation is involved. No fixation step is needed. Simply rinse gel, add RAPIDstain™ and protein bands develop within 5-10 minutes, reaching a maximum visibility in an hour. Mini-gels can be stained in ten minutes.

RAPIDstain™ only stains protein, leaving a crystal-clear background resulting in high band visibility. The gels require no destaining. The protein band intensity is enhanced by simply rinsing with water.

RAPIDstain™ has the sensitivity to detect as little as 4-8ng BSA. Staining shows a linear response for densitometric gel analysis and produces sharp scanning or photographic results.

It is 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 4: Polyacrylamide gel stained with RAPIDstain™.

FEATURES

- Sensitive, 4-8ng BSA
- Saves time, band develops within 5-10 minutes
- Uses a single step method
- No methanol or acetic acid destaining agents

APPLICATIONS

- Staining of protein electrophoresis gels
- For native, denaturing, isoelectric focusing, and 2D gels

CITED REFERENCES

Nalvarte, I. et al (2010) *Mol. Cell. Proteomics.* 9:1411
Li, D. et al (2010) *J. Cell Sci.* 123:2008
Mohan, S. et al (2009) *Am J Physiol Cell Physiol.* 296: C182
Bostick, B. et al (2008) *Circ. Res.* 102: 121
Caswell, C.C. et al (2007) *Mol Microbiol.* 64:1319
Han, R. et al (2006) *Mol Microbiol.* 61:351
Han, R. et al (2006) *Appl Microbiol Biotechnol.* 72:109

Cat. #	Description	Size
786-31	RAPIDstain™	1L for 50 mini gels
786-31G	RAPIDstain™	1gal for ~200 mini gels

Coomassie Brilliant Blue

Coomassie Brilliant Blue R-250/ G-250 are both offered as dry power form and convenient ready-to-use solutions. Destaining solution is also offered.

Cat. #	Description	Size
786-495	Coomassie Brilliant Blue R-250 Dye	10g
786-496	Coomassie Brilliant Blue G-250 Dye	10g
786-497	Coomassie Brilliant Blue G-250 Solution	1L
786-498	Coomassie Brilliant Blue R-250 Solution	1L
786-499	Coomassie Brilliant Blue Destaining Solution	1L

SILVER STAINS

FASTsilver™

A rapid silver stain for proteins

A nanogram sensitive silver staining kit that produces crystal clear background and maximal sensitivity needed for critical analysis.

A unique formulation that leaves the background clear and produces sharp images of protein bands. FASTsilver™ detects as little as 1 ng BSA protein.

FASTsilver™ does not use the protein modifier glutaraldehyde and therefore allows for the complete recovery of proteins and trypsin digested peptides.

It also stains nucleic acids and is able to detect as little as 0.3ng.

The kit contains ready-to-use reagents for 25 mini gels and comes with a simple to follow 60-90 minute protocol.

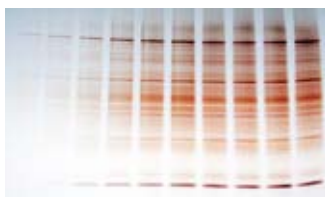


Figure 5: Tissue extract loaded on 4-20% gradient gel and developed with FASTsilver™. Protein loaded; 0.25µg to 5µg per well.

FEATURES

- Stains both proteins and nucleic acids
- Produces clear background for maximum visibility
- Protocol time 60-90 minutes

APPLICATIONS

- Staining of proteins and nucleic acids in electrophoresis gels
- For native, denaturing, isoelectric focusing and 2D gels

CITED REFERENCES

Reed, D. et al (2005) *Plant Cell Reports*. 24:15
 Ortega, N. et al (2005) *Mol. Biol. Cell* 16: 3028
 Venkatesh, S.G. et al (2004) *Am. J. Physiol. Cell Physiol.* 286: C365
 Melody, J. L. et al (2004) *J. Anim. Sci.* 82: 1195
 Delcroix, J. D. et al (2003) *Neuron* 39: 69
 Wu, C. et al (2003) *Am. J. Respir. Cell Mol. Biol.* 2: 731
 Kralj, S. et al (2002) *Appl. Envir. Microbiol.* 68: 4283

Cat. #	Description	Size
786-30	FASTsilver™ Kit	25 Mini Gels

FOCUS™ FASTsilver™

Mass spectrometry compatible silver stain

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

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 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 6: 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 <90 minutes

Cat. #	Description	Size
786-240T	FOCUS™ FASTsilver™ Kit	5 mini Gels
786-240	FOCUS™ FASTsilver™ Kit	25 mini Gels

Silver D-Stain™

Destain & restain your protein or nucleic acid gels

The staining of gels with silver stains often results in over staining or unevenly stained gels. Silver D-Stain™ saves the trouble of repeating the electrophoresis.

FEATURES

- Reduces staining of silver stained gels to reveal over stained bands
- Destain gels completely and restain or follow other downstream applications, such as Western transfer or sequencing. Suitable for both protein and nucleic acid gels



Figure 7: Top lane shows a gel over stained with FASTsilver™ and the bottom lane shows the same gel destained with Silver D-Stain™ and then restained with FASTsilver™, this allows more protein bands to be visualized.

Cat. #	Description	Size
786-199	Silver D-Stain™	25 Mini Gels

REVERSIBLE STAINS

Reversible Copper Stain™

The *Reversible Copper Stain™* is a single step stain for rapid detection of proteins resolved on SDS-PAGE. No destaining is necessary.

The stain is based on the interaction of copper ions with polyacrylamide and proteins. The stain works by depositing a copper metal precipitate in the gel, resulting in an opaque blue/green gel, while the SDS coating on the proteins inhibits copper ions from binding to the proteins. A negative image of the gel is produced; consisting of clear protein bands visualized against a semi-opaque blue/green polyacrylamide background.

Protein bands are visualized in as little as 5 minutes. The sensitivity of the *Reversible Copper Stain™* is 0.1-0.5ng BSA protein. Staining does not interfere with the electroelution of proteins or alter their biological properties. Gels stained with the *Reversible Copper Stain™* can be destained in 20-25 minutes before the transfer or electroelution of proteins. Not suitable for native gels or gels containing Tricine or Glycine.

The kit contains ready to use reagents for 25 mini gels and is supplied with a destainer. A larger size of the destain for the *Reversible Copper Stain™* is offered for your convenience.



Figure 8: Polyacrylamide gel stained with Reversible Copper Stain™.

FEATURES

- Stains gels within 5 minutes
- Reversible, simple and fast stain
- Allows subsequent elution, blotting, or other types of staining

APPLICATIONS

- Suitable for staining of protein gels prior to transfer or electroelution of proteins

Cat. #	Description	Size
786-32CU	Reversible Copper Stain™	25 mini gels
786-32DSCU	Destaining Solution- Copper [10X]	500ml

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 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™* 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 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™*. Phosvitin, a phosphoglycoprotein, was resolved on two gels and stained either with a silver stain or *Reversible Zinc Stain™*. The phosvitin was only detected with the *Reversible Zinc Stain™*.

FEATURES

- Reversible, simple, fast, and sensitive
- High contrast protein bands
- For proteins difficult to detect with silver stains, i.e. glycoproteins

APPLICATION

- 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 9: 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.

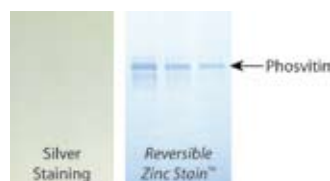


Figure 10: Phosvitin, a phosphoglycoprotein, was separated by SDS-PAGE and developed either by a silver staining method or the Reversible Zinc Stain™. Phosvitin was only visualized with the Reversible Zinc Stain™.

Cat. #	Description	Size
786-32ZN	Reversible Zinc Stain™	25 mini gels
786-32DSZN	Destaining Solution-Zinc [10X]	500ml

GLYCOPROTEIN STAINING

For staining protein gels for glycoproteins

For the highly sensitive detection of glycoproteins following gel electrophoresis (Figure 11A & B) or protein transfer to nitrocellulose membranes (Figure 11C).

The kit uses an enhanced Periodic Acid-Schiff (PAS) method for detection of glycoprotein sugars. The supplied oxidizing agent first oxidizes the cis-diol sugar groups to aldehydes. The aldehyde groups react with the sensitive Glyco-Stain Solution forming Schiff bonds and producing strong magenta color bands.

In addition to glycoprotein staining, the kit is supplied with RAPIDstain™, an enhanced Coomassie stain. RAPIDstain™ can be used after glycoprotein staining to detect non-glycosylated proteins and the use of the stain enhances glycoprotein staining (Figure 11B).

The Glycoprotein Staining kit is highly convenient as all the key reagents required for staining are supplied and a unique positive & negative control is included. In addition, the kit allows for the detection of glycosylated and non-glycosylated proteins on a single gel or membrane.

The kit is sufficient for 10 mini gels (8 x 8cm) or 20 nitrocellulose membrane (8 x 8cm).

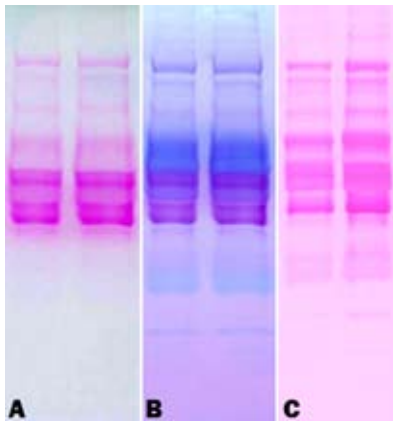


Figure 11: Glycoprotein Staining Kit positive control was resolved on a 4-20% SDS polyacrylamide gel. A. The gel was stained with by the Glycoprotein Staining kit and then with the included RAPIDstain™ (B). A separate gel was transferred to a nitrocelluole membrane and the membrane then stained with the Glycoprotein Staining kit (C).

FEATURES

- Specific glycosylated protein staining with optional non-glycosylated protein staining
- Two stains in one kit
- Improved detection of glycoproteins
- Nanogram level sensitivity
- Stable periodate/ Schiff staining

APPLICATIONS

- Detection of glycoproteins in polyacrylamide gels or on nitrocellulose membranes

Cat. #	Description	Size
786-254	Glycoprotein Staining Kit	10 mini gels

GEL STAIN SELECTION GUIDE

	Sensitivity [BSA] (ng)	Staining Time (min)	Application Comments
LabSafe GEL Blue™	4-8	3-60	<ul style="list-style-type: none"> • Improved Coomassie based stain • No destaining required • Native, denaturing, IEF & 2D gels
RAPIDstain™	4-8	5-60	<ul style="list-style-type: none"> • Improved Coomassie based stain • No destaining required • Native, denaturing, IEF & 2D gels
Coomassie Brilliant Blue	8-10	60	<ul style="list-style-type: none"> • Destaining required • Colloidal Coomassie stain
FASTsilver™	0.5-1.0	60-90	<ul style="list-style-type: none"> • Stains proteins and nucleic acids • Native, denaturing, IEF & 2D gels
FOCUS FASTsilver™	0.5-1.0	60-90	<ul style="list-style-type: none"> • Mass Spectrometry compatible • Allows proteolytic digestion • For complete peptide extraction • Native, denaturing, IEF & 2D gels
Reversible Copper Stain™	0.1-0.5	5-10	<ul style="list-style-type: none"> • Reversible • Compatible with subsequent applications, including elution and transfers. • Not compatible with native gels or gels containing tricine or glycine
Reversible Zinc Stain™	0.1-0.5	10-20	<ul style="list-style-type: none"> • Reversible • Stains glycoproteins, phosphoproteins & other problematic proteins • Compatible with subsequent applications, including elution and transfers • Native and SDS denatured gels and gels containing glycine and tricine
Glycoprotein Staining Kit	0.5-1.0	180	<ul style="list-style-type: none"> • Combined dye and silver stain kit • Improved glycoprotein detection • Native, denaturing, IEF & 2D gels

MILD EXTRACTION BUFFERS

Protein Extraction & Lysis Buffer (PE LB™) Systems

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

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

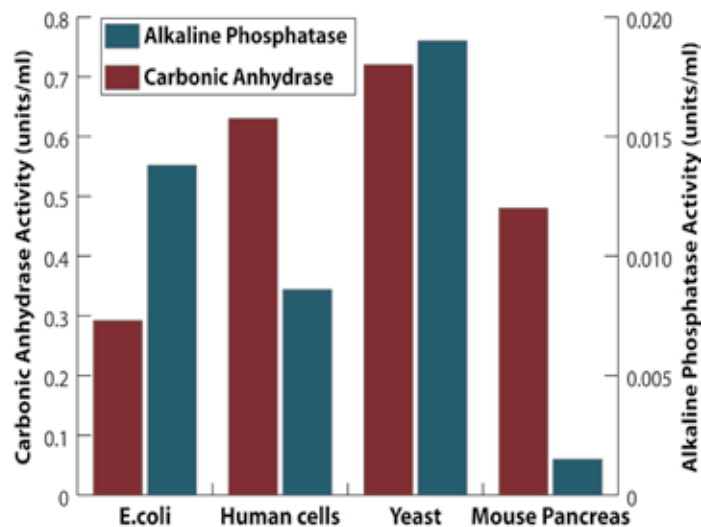


Figure 14: PE LB™ System maintains the biological activity of proteins. Extraction of carbonic anhydrase or alkaline phosphatase from E.coli, human cells, yeast and mouse pancreas with Bacterial, Mammalian Cell, Yeast and Tissue PE LB™ respectively. The resulting lysates were submitted to enzyme assays and both enzymes retain their biological activity.

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

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

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

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.

Bacterial PE LB™

Extraction of bacterial and recombinant proteins

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

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

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

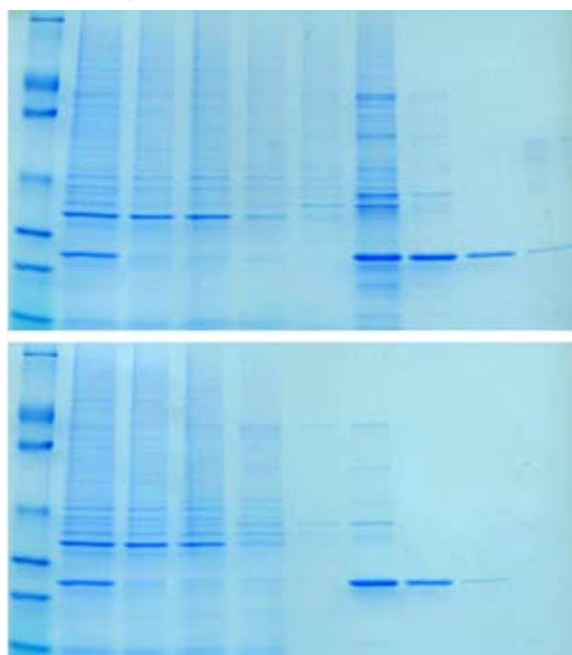


Figure 15: Bacteria expressing a His-tagged protein were lysed with Bacterial PE-LB™ and the recombinant protein was purified with HOOK™ 6X His Protein Purification kits (Top: Nickel resin; Bottom: Cobalt resin). Lane 1: PAGEDmark™ protein ladder; 2: Cleared lysate; 3: Flow through; 4-6: Washes; 7-9: Elutions.

FEATURES

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

APPLICATIONS

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

Cat. #	Description	Size
786-176	Bacterial PE LB™ Kit including PE LB™ Lysozyme	100 preps
786-177	Bacterial PE LB™ buffer only	500ml

Yeast PE LB™

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

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

FEATURES

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

APPLICATIONS

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

CITED REFERENCES

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

Cat. #	Description	Size
786-178	Yeast PE LB™ Kit including Zymolyase®	100 preps
786-179	Yeast PE LB™, buffer only	500ml

Mammalian Cell PE LB™

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

FEATURES

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

APPLICATIONS

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

CITED REFERENCES

Yu, J., et al (2008) *PNAS*. 105: 19300
 Zhang, L., et al (2006) *Cancer Gene Ther.* 13: 74
 Valverde, P., et al (2004) *Exp Eye Res.* 78: 27
 Qin, M., et al (2003) *Clin. Cancer Res.* 9: 4992

Cat. #	Description	Size
786-180	Mammalian Cell PE LB™	500ml

Tissue PE LB™

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

FEATURES

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

APPLICATIONS

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

CITED REFERENCES

Kavanaugh, K. et al (2011) *Am J Physiol Endocrinol Metab.* 300:E894-E901
 Ray, S. et al (2008) *Mol. Endocrinol.* 22: 1125
 Stein, D. et al (2008) *J. Antimicrob. Chemother.* 62: 555
 Wang, Z. et al (2007) *J. Neurosci.* 27: 3686
 Yoshino, O. et al (2006) *PNAS* 103: 10678
 Shariat-Madar, Z et al (2006) *Blood* 108: 192
 Yao, L et al (2005) *Blood* 106:13
 Mangino, M. et al (2004) *Am. J. Physiol. Renal Physiol.* 286: F838

Cat. #	Description	Size
786-181	Tissue PE LB™	500ml

Insect PE LB™

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

FEATURES

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

APPLICATIONS

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

Cat. #	Description	Size
786-411	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. Cyanate reacts with α-amino and ε-amino groups of proteins and induces a change in the isoelectric point of proteins. This leads to artifactual results and therefore carbamylation must be avoided.

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

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

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

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

For analysis of a proteome, a single buffer may not be suitable and sequential solubilization using different FOCUS™ Extraction Buffers will help in identifying new proteins. **“Improved Protein Extraction for 2D Electrophoresis by Sequential Solubilization with FOCUS™ Chaotropic Extraction Buffers”** is available at GBiosciences.com.

FEATURES

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

APPLICATIONS

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

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

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

Cat. #	Description	Size
786-220	FOCUS™ Extraction Buffer I	For 50ml
786-221	FOCUS™ Extraction Buffer II	For 50ml
786-222	FOCUS™ Extraction Buffer III	For 50ml
786-223	FOCUS™ Extraction Buffer IV	For 50ml
786-219	FOCUS™ Extraction Buffer V	For 50ml
786-233	FOCUS™ Extraction Buffer VI	For 50ml
786-234	FOCUS™ Extraction Buffers I-VI Trial kit	For 10ml each buffer

A range of FOCUS™ Extraction Buffers, listed with their major components, are shown. 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 more and new proteins. The application note **“Improved Protein Extraction for 2D Electrophoresis by Sequential Solubilization with FOCUS™ Chaotropic Extraction Buffers”** is available at www.GBiosciences.com.

FEATURES

- Convenient and simple to use extraction buffers
- Simply hydrate and use
- Prevents urea induced protein carbamylation
- Prevents waste of unused reagents
- No artifactual protein bands due to dust and human skin contamination

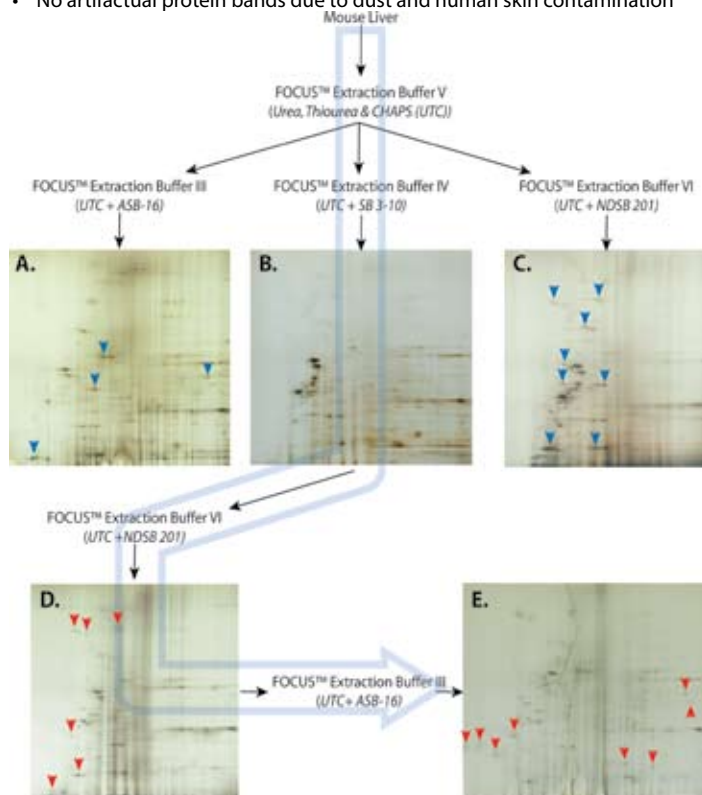


Figure 16: Serial Solubilization of Mouse Liver. Mouse liver was solubilized in FOCUS™ Extraction Buffer V and the insoluble material was further solubilized. The insoluble pellet was solubilized in either FOCUS™ Extraction Buffer III (A), IV (B) or VI (C) and the proteins were resolved by 2D electrophoresis. The blue arrowheads highlight a selection of different proteins compared to gel B. In a second analysis, the insoluble pellet from FOCUS™ Extraction Buffer V was serially extracted in FOCUS™ Extraction Buffer IV (B), then VI (D) and finally III (E). These were resolved by 2D electrophoresis. New proteins appearing at each stage are indicated with red arrows. UTC= Urea, thiourea & CHAPS.

2D-Xtract™

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

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

FEATURES

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

APPLICATIONS

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

Cat. #	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.

Cat. #	Description	Size
786-246	FOCUS™ Mammalian Proteome	50 preps
786-360	FOCUS™ Insect Proteome	50 preps
786-257	FOCUS™ Yeast Proteome	50 preps
786-258	FOCUS™ Bacterial Proteome	50 preps
786-259	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.

Cat. #	Description	Size
786-225	Total Protein Extraction (TPE™) Kit	50 preps

RIPA Lysis & Extraction Buffer

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

Cat. #	Description	Size
786-489	RIPA Lysis & Extraction Buffer	100ml
786-490	RIPA Lysis & Extraction Buffer	500ml

Inclusion Body Solubilization Buffers

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

Cat. #	Description	Size
786-183	IBS™ Buffer Kit	100ml
786-183HP	IBS-HP™ Buffer Kit	100ml

DETERGENTS

Proteomic Grade Detergent Solutions (10%)

Ultra low carbonyl & peroxide contaminants

Many commercial grade detergents contain elevated levels of sulfhydryl oxidizing agents, peroxides, salts and carbonyl compounds. The proteins that are isolated with these detergents are highly susceptible to contaminating peroxides and carbonyls. The peroxides will oxidize proteins and the carbonyl groups will form Schiff's bases with the proteins that will interfere with a protein's structure.

Our Proteomic Grade Detergent Solutions contain reduced peroxides and carbonyl compounds. In addition, the detergents have less than 50 μ S conductivity. These detergents are offered as 10% aqueous solutions, sealed under inert gas and are suitable for protein applications. These non-ionic detergents are suitable for isolating membrane-protein complexes.

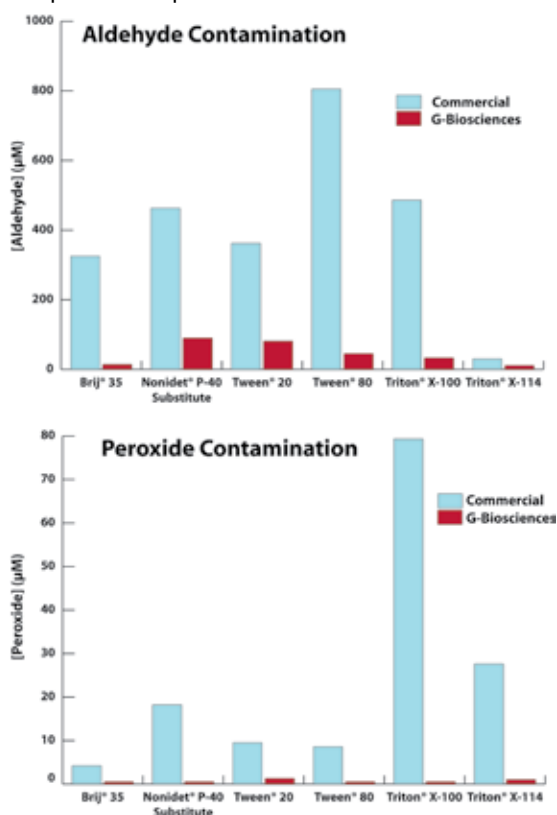


Figure 17: Comparison of aldehyde (top) and peroxide (bottom) concentration in G-Biosciences Proteomic Grade Detergent Solutions and non-proteomic grade commercially available detergents.

FEATURES

- Low peroxide contamination
- Low carbonyl contamination
- Low conductivity
- Reduced metal ions
- 10% aqueous solutions
- Sealed under inert gas to prevent oxidation

For a complete selection of our detergents download the "Detergent Handbook and selection guide".

PROTEASE INHIBITOR COCKTAILS

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 10 μ l 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 and protein purification from animal tissues, plants, yeast and bacteria.

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

ProteaseArrest™ Outperforms Tablet Cocktails

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

In our study, a 1X concentration of ProteaseArrest™ inhibits over 95% of protease activities (e.g. 0.5mg/ml mouse pancreas extract). The ProteaseArrest™ protease inhibitor cocktail demonstrated greater inhibition levels compared to similar protease inhibitor cocktails, including tablet formats (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
- Protection of proteins from proteolysis in such applications as electrophoresis, purification, storage, assays, and other applications

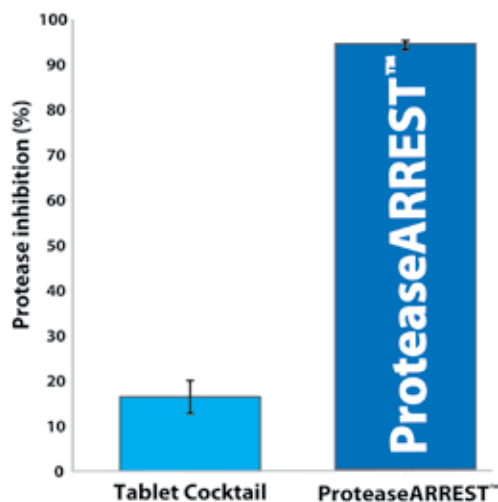


Figure 18: ProteaseArrest™ outperforms tablet format protease inhibitor cocktails. Protease inhibition in mouse pancreas lysate with ProteaseArrest™ (EDTA-free) and a commercially available EDTA-free tablet protease cocktail was compared, using Protease Screening™ Kit. The assay used 0.5mg/ml pancreas lysate and incubation conditions of 37°C for 2.5 hours. ProteaseArrest™ inhibited over 95% of total proteases, 80% more compared to tablet inhibition.

PHOSPHATASE INHIBITOR COCKTAILS

The PhosphataseArrest™ 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

PhosphataseArrest™ I

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

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

CITED REFERENCES

1. Garrido-Lecca, A. and Blumenthal, T. (2010) *Mol. Cell. Biol.* 30:3887-3893

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 100X solution of cantharidin, bromotetramisole, and microcystin LR.

PhosphataseArrest™ IV

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

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

CITED REFERENCES

- Orkwis, B.R. et al (2010) *Genetics* 186: 885
 Sekar, Y et al (2010) *J. Immunol.* 185:578
 Niamh, X et al (2010) *Am J Physiol Endocrinol Metab.* 299:E189
 Roth, K. et al (2009) *Int. Immunol.* 21: 19
 McMahon, H. et al (2008) *Endocrinology.* 149: 812
 Ram, E. V. S. et al (2008) *Nucleic Acids Res.* 36: 5061
 Kitareewan, S. et al (2007) *JNCI.* 99:41
 Gennidakis, S. et al (2007) *Plant J.* 52: 839
 Gu, T. et al (2006) *Blood.* 108:4202
 Timney, B. et al (2006) *J. Cell Biol.* 175: 579
 Rocnik, J. et al (2006) *Blood.* 108: 1339

Cat. #	Description	Size
786-108	ProteaseArrest™ [100X]	2ml
786-329	OneQuant™ ProteaseArrest™ [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.

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

Cat. #	Description	Size
786-108F	FOCUS™ ProteaseArrest™ [100X]	1ml

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.

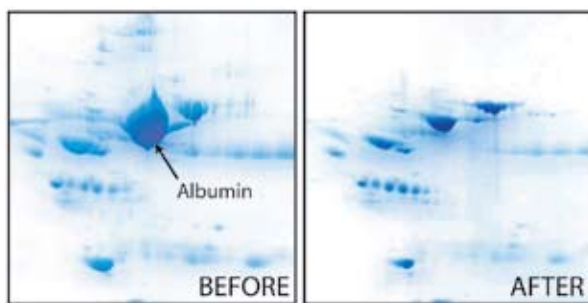


Figure 19: 2D analysis of whole human serum before (left) and after (right) treatment with AlbuminOUT™.

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

Cat. #	Description	Size
786-251	AlbuminOUT™	25 preps
786-252	AlbuminOUT™	50 preps

IMMUNOGLOBULIN G REMOVAL

Immobilized Protein A

For binding the constant domains of immunoglobulin (Ig) molecules. Protein A is coupled to agarose beads by a proprietary coupling method that provides high coupling efficiency for immunoglobulins and minimal protein A leaching. Immobilized Protein A Resin is available as resin alone or supplied in a kit format containing:

- 5ml resin
- 100ml Protein A or G Binding/Wash Buffer (0.1M sodium phosphate, 0.15M NaCl, pH7.5)
- 100ml Protein A or G Elution Buffer (100mM glycine, pH3.0)
- 5 empty 1ml spin columns
- 5 empty <5ml gravity flow columns

The buffers are also available separately.

FEATURES

- For the binding of immunoglobulins
- High binding capacity: 18-43mg/ml resin
- Bead Structure: 6% highly cross-linked agarose

APPLICATIONS

- For immunoaffinity chromatography & immunoprecipitation

Cat. #	Description	Size
786-283	Immobilized Protein A Resin	5ml resin
786-553	Immobilized Protein A Resin Kit	1
786-544	Protein A or G Binding/ Wash Buffer	100ml
786-545	Protein A or G Elution buffer	100ml

Immobilized Protein G

For binding the constant domains of immunoglobulin (Ig) molecules. Protein G is a modified form of Streptococcal group G so that it does not bind to albumin. Protein G is coupled to 4% cross-linked agarose beads by a proprietary coupling method that provides high coupling efficiency for Ig and minimal protein G leaching. Immobilized Protein G Resin is available as resin alone or supplied in a kit format containing:

- 5ml resin
- 100ml Protein A or G Binding/Wash Buffer (0.1M sodium phosphate, 0.15M NaCl, pH7.5)
- 100ml Protein A or G Elution Buffer (100mM glycine, pH3.0)
- 5 empty 1ml spin columns
- 5 empty <5ml gravity flow columns

The buffers are also available separately.

FEATURES

- For the binding of immunoglobulins
- High binding capacity: >20mg Human IgG/ml resin
- Ligand density: ~2mg protein G /ml resin
- Bead size: 50-160µm
- Bead Structure: 4% highly cross-linked agarose

APPLICATIONS

- For immunoaffinity chromatography & immunoprecipitation

Cat. #	Description	Size
786-284	Immobilized Protein G Resin	5ml resin
786-554	Immobilized Protein G Resin Kit	1
786-544	Protein A or G Binding/ Wash Buffer	100ml
786-545	Protein A or G Elution buffer	100ml

Glycoproteins

FOCUS™ 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.

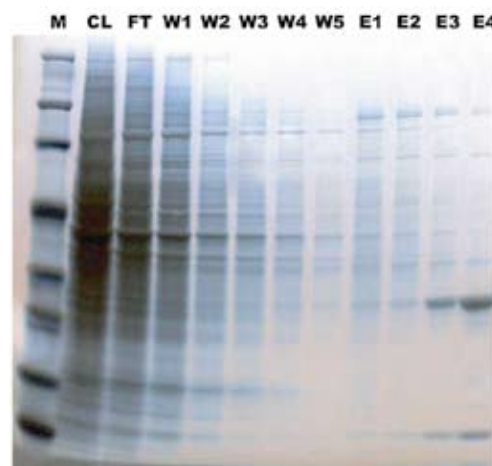


Figure 21: FOCUS™ Glycoprotein isolates multiple glycoproteins. Jurkat cells were lysed by sonication, centrifuged and the supernatant (CL) loaded onto a FOCUS™ Glycoprotein column. The column was centrifuged and the flow through (FT) collected. The column was washed (W1-5) and the glycoproteins were eluted with Glyco Elution Buffer I (E1-2), Glyco Elution Buffer II (E3) and then Glyco Elution Buffer III (E4). 10µl 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

Cat.#	Description	Size
786-253	FOCUS™ Glycoprotein	10 Preps

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

FEATURES

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

APPLICATIONS

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

Cat.#	Description	Size
786-255	FOCUS™ PhosphoRich™	5 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.

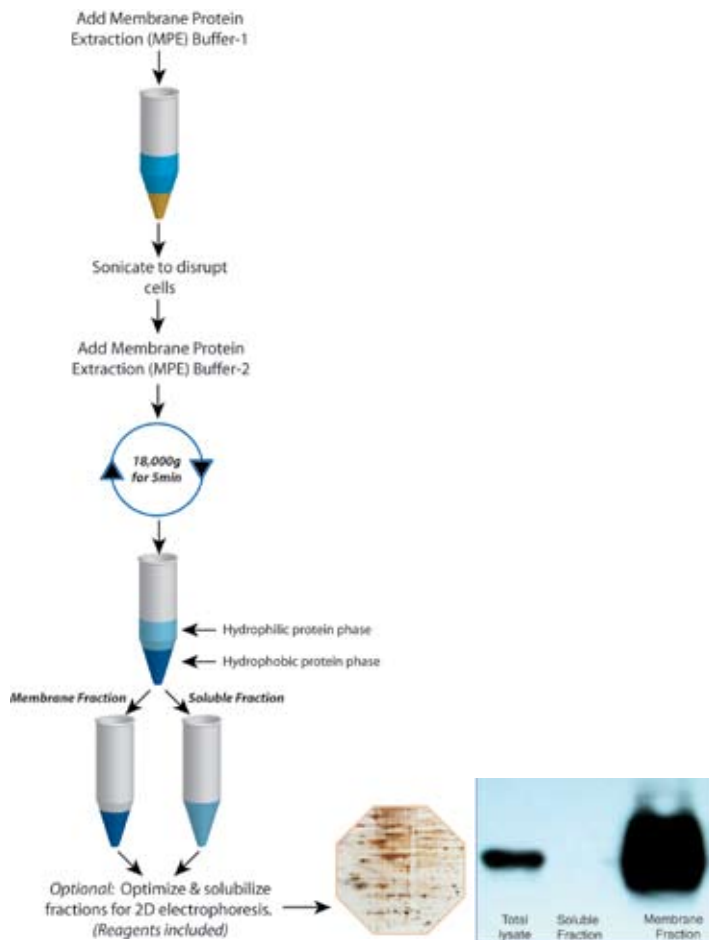


Figure 22: (Left) FOCUS™ Membrane Protein scheme. (Right) Mouse liver was processed with FOCUS™ Membrane Proteins kit. The enriched membrane and soluble fractions were resolved by SDS-PAGE, transferred and probed with antibodies against caveolin, a membrane protein.

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
- Suitable for membrane protein preparation for a wide range of downstream applications, including 1D & 2D electrophoresis, Western blotting and mass spectrometry

Cat.#	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

APPLICATIONS

- For cell surface trafficking & receptor:ligand interactions

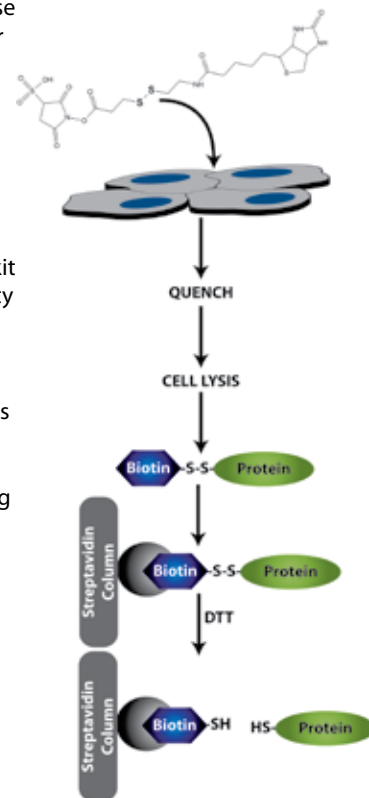


Figure 23: HOOK™ Cell Surface Protein Isolation scheme.

Cat.#	Description	Size
786-316	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 glycosyl-phosphatidylinositol. Lipid rafts are also known as detergent-insoluble glycolipid-enriched complexes (GEMs) or DIGs. Many signaling proteins, including glycosylphosphatidylinositol (GPI)-anchored proteins, doubly-acylated tyrosine kinases of the Src family, and transmembrane proteins, are located in lipid rafts. Lipid raft localized proteins have been shown to be involved in intracellular membrane trafficking and signaling.

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

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

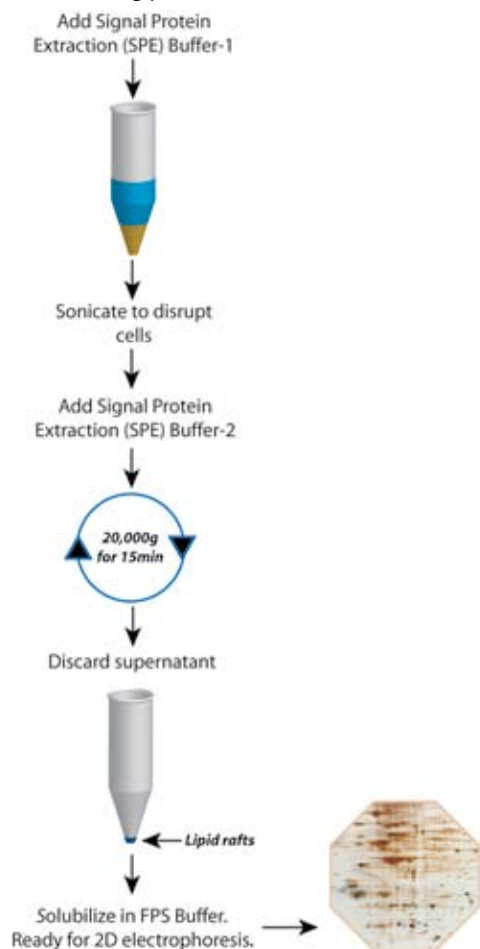


Figure 24: FOCUS™ Signal Protein scheme.

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

Cat.#	Description	Size
786-250	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

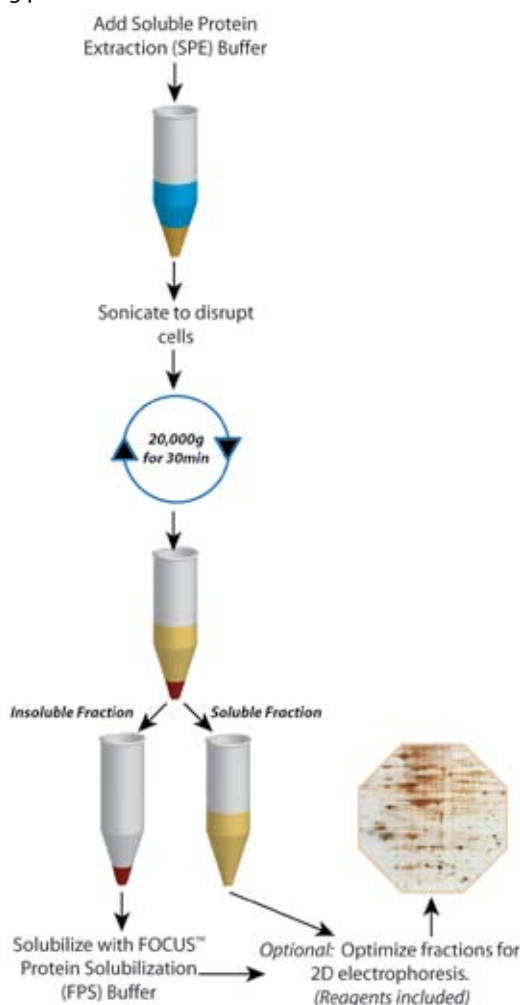


Figure 25: FOCUS™ Soluble & Insoluble scheme.

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.#	Description	Size
786-247	FOCUS™ Soluble & Insoluble Kit	50 Preps

For further details, visit www.GBiosciences.com

Protein Fractionation & Enrichment

FOCUS™ Global Fractionation

Enrichment of cytosolic and membrane signal, peripheral or integral proteins

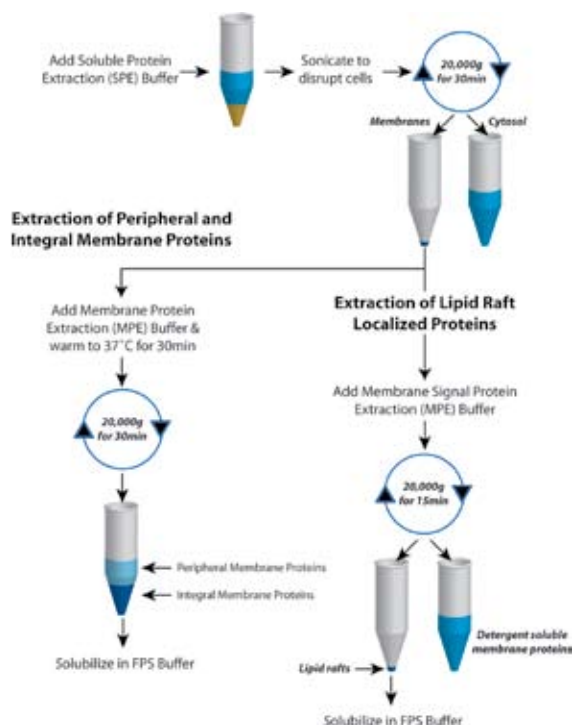


Figure 26: FOCUS™ Global Fractionation scheme.

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 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.

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

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

Cat. #	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 20x10⁶ mammalian cells or 100mg mammalian tissue.

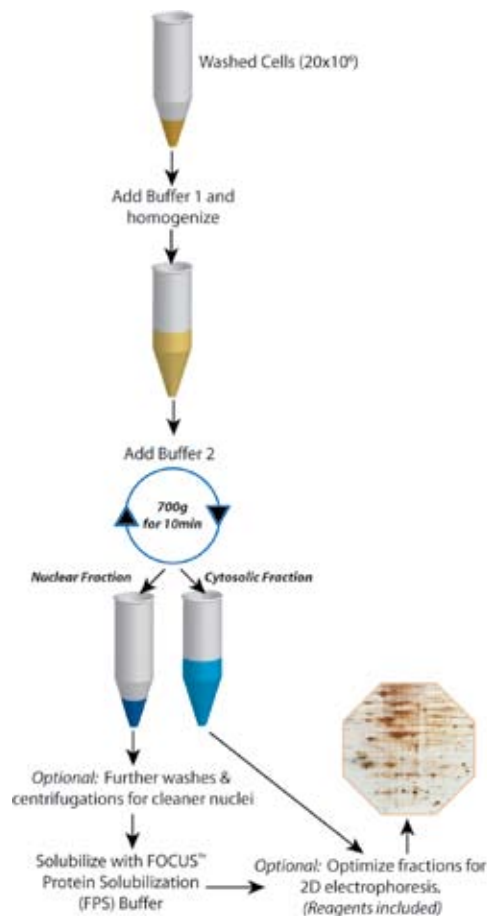


Figure 27: FOCUS™ Cytoplasmic & Nuclear Protein scheme.

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

Cat.#	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 2×10^7 mammalian cells or 100mg mammalian tissue.

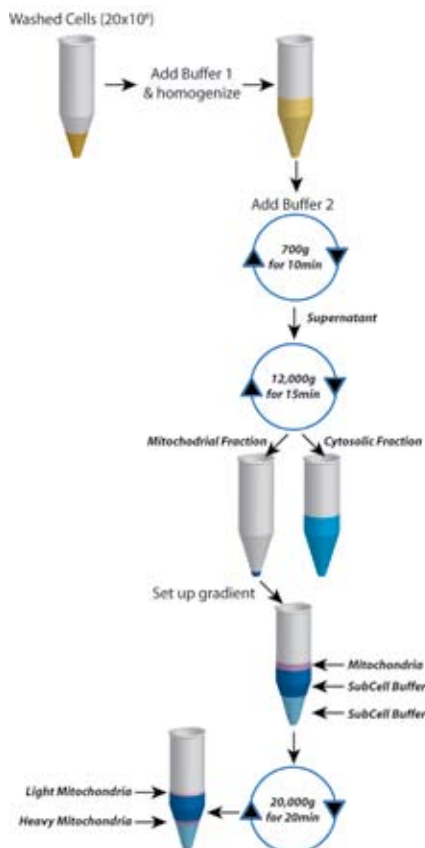


Figure 28: FOCUS™ Mitochondria scheme.

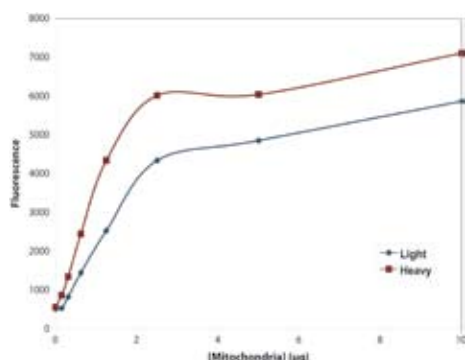


Figure 29: Activity of mitochondria purified with FOCUS™ Mitochondria, as determined with a JC-1 Assay.

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

Cat. #	Description	Size
786-022	FOCUS™ Mitochondria Kit	50 Preps

Subcellular Fractionation

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

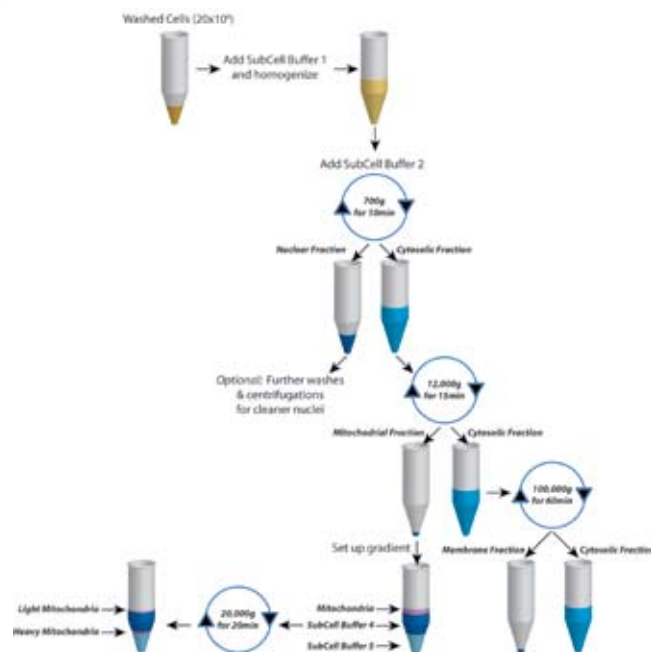


Figure 30: FOCUS™ SubCell scheme.

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 2×10^7 mammalian cells or 100mg mammalian tissue.

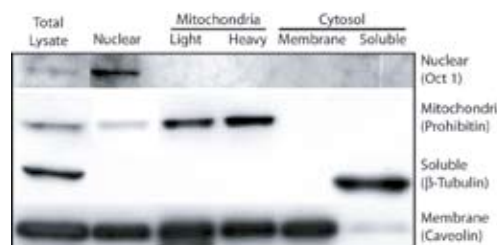


Figure 31: NIH3T3 cells were fractionated with FOCUS™ SubCell. The fractions were resolved by SDS-PAGE and then transferred to a PVDF membrane. The membrane was probed with the indicated antibodies.

FEATURES

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

APPLICATIONS

- For mitochondrial, nuclear, cytosolic and membrane fractions

CITED REFERENCES

- Upreti, M. et al (2008) Mol. Cancer Ther. 7: 2224
 Son, M. et al (2007) PNAS. 104: 6072

Cat. #	Description	Size
786-260	FOCUS™ SubCell Kit	50 Preps

GENERAL FRACTIONATION

Fraction-FOCUS™

Fractionation for cleaner 2D gel maps

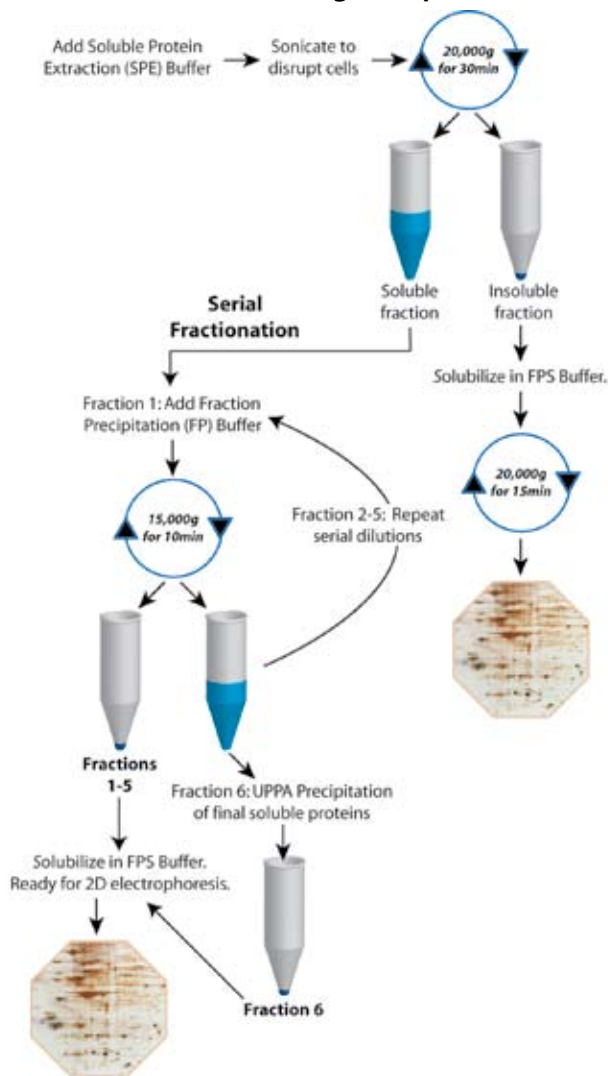


Figure 32: Fraction-FOCUS™ scheme.

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

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

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

There is no detectable loss of total protein during the Fraction-FOCUS™ 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

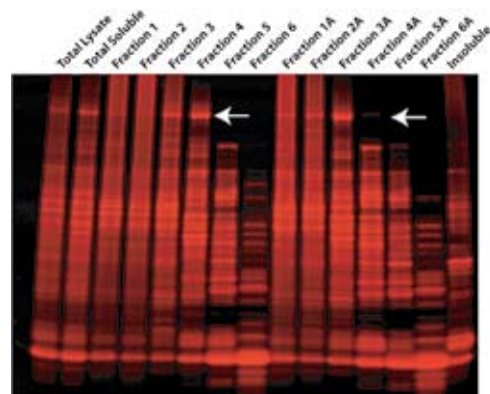


Figure 33: Mouse liver lysate was analyzed with Fraction-FOCUS™ (Fractions 1-6, normal protocol; Fractions 1A-6A, alternative protocol). 5µg protein was loaded on a 4-20% SDS polyacrylamide gel and stained with a fluorescent stain. Fraction-FOCUS™ simplifies the protein maps, compared to total lysate, 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.#	Description	Size
786-168	Fraction-FOCUS™	10 Preps

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.

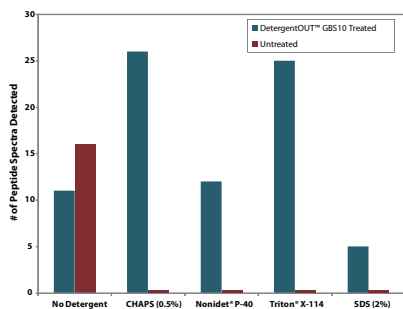


Figure 34: DetergentOUT™ GBS10 removes detergent and allows detection of peptide fragments by Mass spectrometry. 500µg phosphorylase B was digested in solution and then the indicated amount of detergent was added. Samples were treated with DetergentOUT™ GBS10. Number of peptide spectra were determined as per the protocol of Alvarez, S. et al.

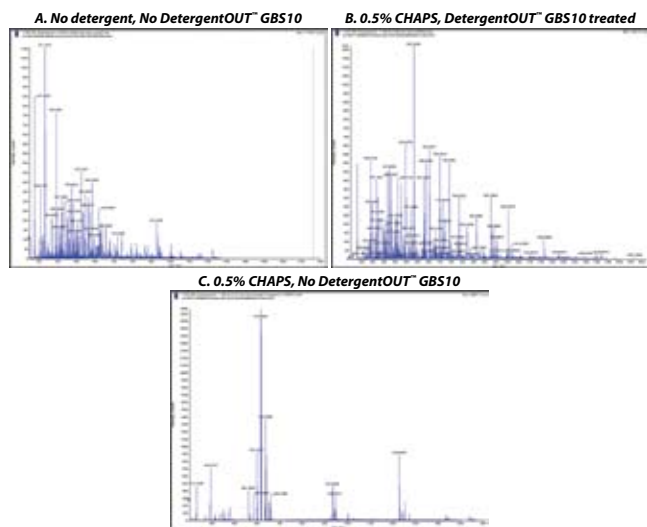


Figure 35: DetergentOUT™ GBS10 enhances Mass spectrometry Spectra. 5µg/µl protein mixture (BSA, cytochrome C and phosphorylase B) in water (Panel A) was supplemented with 0.5% CHAPS (Panel B and C). The CHAPS containing sample was treated with DetergentOUT™ GBS10 and compared to an untreated sample (Panel C). Spectra were generated per Alvarez et al.

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

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

REFERENCES

Alvarez, S. et al (2010) Poster presented as part of the 58th ASMS Conference on Mass Spectrometry and Allied Topics, May 23-27, 2010, Salt Lake City, Utah

Cat. #	Description	Sample Size (µl)	Resin (µl)	Size
786-154	DetergentOUT™ GBS10-125	10-30	125	10 columns
786-155	DetergentOUT™ GBS10-800	30-200	800	10 columns
786-156	DetergentOUT™ GBS10-3000	200-750	3,000	10 columns
786-157	DetergentOUT™ GBS10-5000	500-1,250	5,000	10 columns
786-159	DetergentOUT™ GBS10 Resin	-	-	10ml resin

OrgoSol DetergentOUT™

Suitable for hydrophobic proteins, removes detergents and concentrates protein solutions

OrgoSol DetergentOUT™ is suitable for removal of detergents from protein solutions, including hydrophobic protein solutions and is compatible with all detergent types. Its performance is not dependent on 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.

Cat.#	Description	Size
786-127	OrgoSol DetergentOUT™, Micro	For 10ml
786-128	OrgoSol DetergentOUT™, Medi	For 30ml

DetergentOUT™ Tween®

Removal of Tween® (polysorbate) detergents

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.#	Description	Size
786-214	DetergentOUT™ Tween®, Micro	10 columns
786-215	DetergentOUT™ Tween®, Medi	10 columns

DIALYSIS SYSTEMS

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

Tube-O-DIALYZER™

Efficient dialysis with 100% sample recovery

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

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

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

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

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

APPLICATIONS

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

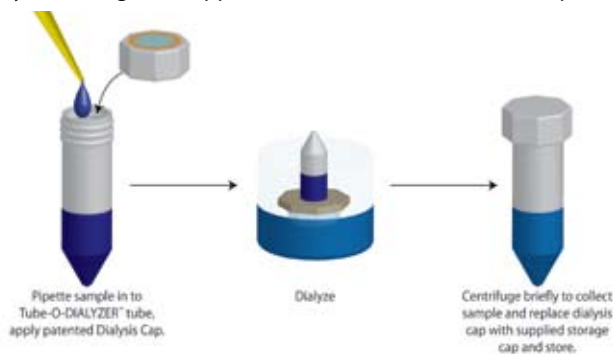


Figure 36: A summary of the Tube-O-DIALYZER™ system.

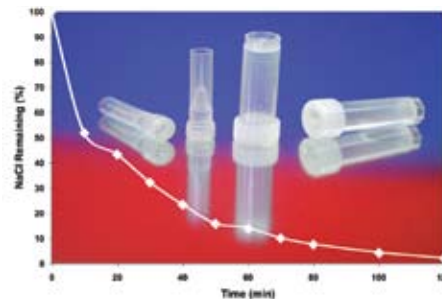


Figure 37: Tube-O-DIALYZER™ micro (8K MWCO) Dialysis Rate. 100µl 5M sodium chloride was dialyzed against 1 liter deionized water. 50% sodium chloride is removed in the first 10 minutes.

CITED REFERENCES

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 Ehmsen, K., et al (2008) *Nucleic Acids Res.* 36: 2182
 Murdoch, A., et al (2007) *Stem Cells.* 25: 2786
 Palmer, C., et al (2008) *PLoS ONE* 3: e2633
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 Thomas, B. and Thekkumkara, T. (2004) *Mol. Biol of the Cell.* 15:4347
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 Kahn, A. et al (2001) *Appl. Envir. Microbiol.* 67: 3577
 Okamoto, H. et al (1998) *Stroke.* 29:1209

Cat. #	Description	Size
786-610	Tube-O-DIALYZER™, Micro, 1k MWCO	20
786-610T	Tube-O-DIALYZER™, Micro, 1k MWCO	5
786-611	Tube-O-DIALYZER™, Micro, 4k MWCO	20
786-611T	Tube-O-DIALYZER™, Micro, 4k MWCO	5
786-612	Tube-O-DIALYZER™, Micro, 8k MWCO	20
786-612T	Tube-O-DIALYZER™, Micro, 8k MWCO	5
786-613	Tube-O-DIALYZER™, Micro, 15k MWCO	20
786-613T	Tube-O-DIALYZER™, Micro, 15k MWCO	5
786-614	Tube-O-DIALYZER™, Micro, 50k MWCO	20
786-614T	Tube-O-DIALYZER™, Micro, 50k MWCO	5
786-615	Tube-O-DIALYZER™, Medi, 1k MWCO	20
786-615T	Tube-O-DIALYZER™, Medi, 1k MWCO	5
786-616	Tube-O-DIALYZER™, Medi, 4k MWCO	20
786-616T	Tube-O-DIALYZER™, Medi, 4k MWCO	5
786-617	Tube-O-DIALYZER™, Medi, 8k MWCO	20
786-617T	Tube-O-DIALYZER™, Medi, 8k MWCO	5
786-618	Tube-O-DIALYZER™, Medi, 15k MWCO	20
786-618T	Tube-O-DIALYZER™, Medi, 15k MWCO	5
786-619	Tube-O-DIALYZER™, Medi, 50k MWCO	20
786-619T	Tube-O-DIALYZER™, Medi, 50k MWCO	5
786-620	Tube-O-DIALYZER™, Mixed, 1k MWCO	20
786-621	Tube-O-DIALYZER™, Mixed, 4k MWCO	20
786-622	Tube-O-DIALYZER™, Mixed, 8k MWCO	20
786-623	Tube-O-DIALYZER™, Mixed, 15k MWCO	20
786-624	Tube-O-DIALYZER™, Mixed, 50k MWCO	20

CONTAMINATION REMOVAL

Spin-OUT™ Desalting Columns

For desalting and buffer exchange

The SpinOUT™ GT-600 and GT-1200 columns are versatile, spin-format columns for the desalting and buffer exchange of protein and nucleic acid solutions ranging from 5µl through to 4ml sample volumes. The SpinOUT™ columns are available in two MWCO sizes. Simply apply the sample and then centrifuge to recover protein/nucleic acids with the column retaining >95% of the salts and small molecules (<1,000Da).

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 >12kDa and nucleic acids larger than 20bp.

Cat. #	Description	Size	Resin Bed (ml)	Sample Load (ml)
786-703	SpinOUT™ GT-600, 0.1ml	25 columns	0.1	0.005-0.02
786-170	SpinOUT™ GT-600, 1ml	10 columns	1	0.05-0.1
786-171	SpinOUT™ GT-600, 3ml	10 columns	3	0.1-0.5
786-704	SpinOUT™ GT-600, 5ml	5 columns	5	0.5-2
786-705	SpinOUT™ GT-600, 10ml	5 columns	10	0.5-4
786-706	SpinOUT™ GT-1200, 0.1ml	25 columns	0.1	0.005-0.02
786-172	SpinOUT™ GT-1200, 1ml	10 columns	1	0.05-0.1
786-173	SpinOUT™ GT-1200, 3ml	10 columns	3	0.1-0.5
786-707	SpinOUT™ GT-1200, 5ml	5 columns	5	0.5-2
786-708	SpinOUT™ GT-1200, 10ml	5 columns	10	0.5-4

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 38: 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.

Cat. #	Description	Size
786-120	UPPA-PROTEIN-Concentrate™ (Micro)	For 10ml sample
786-121	UPPA-PROTEIN-Concentrate™ (Medi)	For 30ml sample
786-122	UPPA™-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.

CITED REFERENCES

Olbro, M. et al (2002) PNAS. 99: 6737

Cat. #	Description	Size
786-125	OrgoSol-PROTEIN-Concentrate™	For 5ml protein

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.

Cat. #	Description	Size
786-126	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; simply 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 250µl (Micro) or 2.5ml (Medi).

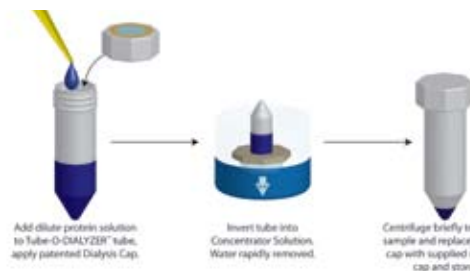


Figure 39: Tube-O-CONCENTRATOR™ scheme.

Cat. #	Description	Size
786-625	Tube-O-CONCENTRATOR™ for 20-250µl	5 concentrations
786-626	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

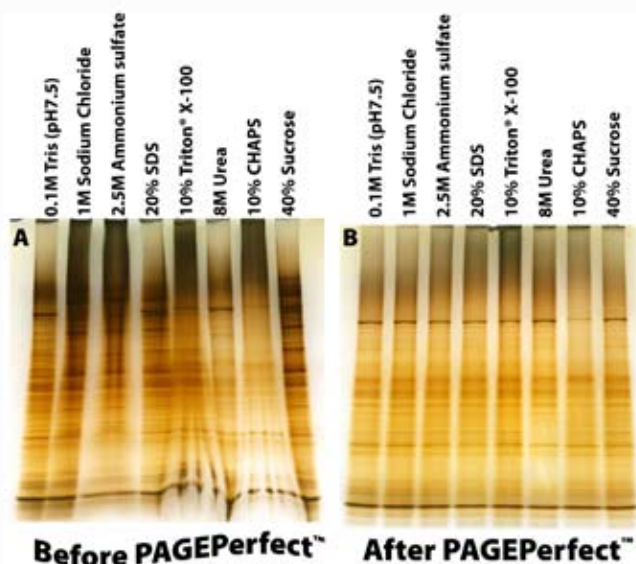


Figure 40: Analysis of mouse liver lysate before and after treatment with PAGE-Perfect™. A. 10µg mouse liver lysates, in the presence of various interfering agents, were loaded onto a SDS polyacrylamide gel. B. 10µg mouse liver lysates, in the presence of various interfering agents, were treated with PAGE-Perfect™ and then loaded onto a SDS polyacrylamide gel. Both gels were stained with FASTsilver™ protein.

Cat. #	Description	Size
786-123	PAGE-Perfect™	50 preps
786-123T	PAGE-Perfect™	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.

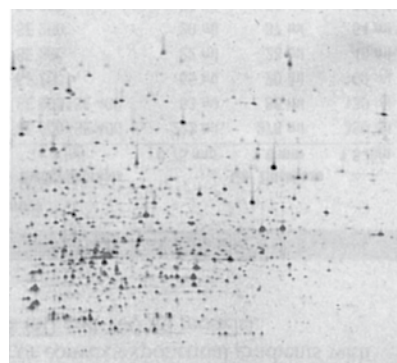


Figure 41: Treatment of protein samples with Perfect-FOCUS™ results in streak free, high spot resolution gels.

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

- Jang, S. et al (2007) JBC. 202: 929
 Laughton, J. et al (2006) Microbiology. 152:1155
 Devillard, E et al (2004) J. Bacteriology. 189: 136
 Rincon, M. et al (2004) J. Bacteriology. 186: 2576
 Jang, S. and Imlay, J. A. (2004) Cancer Research. 64: 9018

Cat. #	Description	Size
786-124	Perfect-FOCUS™	50 preps
786-124T	Perfect-FOCUS™	6 preps

G-Biosciences Product Line Overview

Protein Research

Estimation

7 Assays

Isolation

Extraction & Lysis

Fractionation & Enrichment

Sample Preparation

Reagents

Electrophoresis

Detection

Western Blotting

Mass Spectrometry

Assays (ELISA)

Purification

Affinity Resins

Activated Resins

Antibody Purification

Modification

Labeling

Crosslinkers
Reducing Agents
Alkylating Agents
Protein Cleavage
Iodination
Amino Acid Side Chain Modifiers

Antibody

Production

Purification

Fragmentation

SAM Methyltransferase

Cell Toxicity & Proliferation

Apoptosis

Protease

Phosphatase
Peroxide

B-Galactosidase

Continuous, Enzymatic Assays

Lactate Dehydrogenase (LDH)
SRB
WST-1

Caspase

Inducers
Assays
Inhibitors

CPRG

Fluorescent (MUG)

Genomic DNA

Isolation

Plasmid DNA

Isolation
Colony Screening
Transformation
Apparatus
Loading Dyes
DNA Ladders
Gel Extraction

Electrophoresis

PCR

RNA

Yeast

Tag
dNTPs
Extraction
RNase Decontamination
Transformation
Plasmid Isolation

CB-X
Non Interfering
SPN
RED 660
dotMETRIC
BCA
CB

Sample Grinding

Lysis Buffers

12 Fractionation Kits
Dialysis (Micro)
Concentration

Contamination Removal

Protease Inhibitors

Detergents
Chaotropes

1D & 2D Reagents

Gel Stains

1 Hour System

Blocking Agents

Secondary Antibodies
Chemiluminescence Detection
Trypsin, Mass Spec Grade
InGel Kits
Coated Plates

Blocking Agents

Secondary Antibodies
Detection Reagents

6X His Tag

GST Tag
Biotin Tag
CBP Tag
Sulphydryl reactive
Amine reactive
Carboxyl reactive
Drug/ Steroid reactive
Protein A or G
Pearl Resin
Biotin
Fluorescent Dye
Enzyme (HRP/AP)

Mild Denaturing
Strong Chaotropic
Specialized

Desalting
Detergent Removal
General Cocktails
Species Specific
Individual Inhibitors

2D Specific Kits
Buffers & Reagents
Coomassie
Silver
Reversible

Non-Animal
Animal
Non-Protein

Non-Animal
Animal
Non-Protein

Nickel resin
Cobalt resin
Copper resin
Zinc Resin
Glutathione Resin
Streptavidin Resin
Calmodulin Resin

BSA
KLH
HyperCarrier

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
Bacteria
Fungi
Mouse Tail

