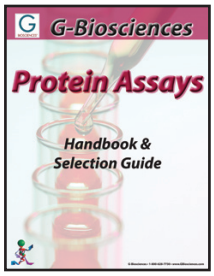
G-Biosciences

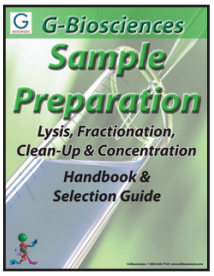
Western Blotting & Electrophoresis

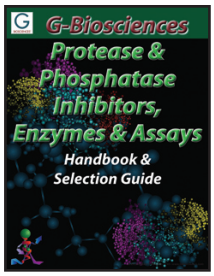
Handbook & Selection Guide



- Protein Estimation Assays
- Apoptosis Assays
- Cytotoxicity Assays
- SAM Methyltransferase Assays
- Protease Assays
- Phosphatase Assays
- Peroxide Assay

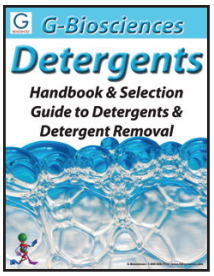
- Lysis Buffers & Systems
- Protein Fractionation Kits
- Dialysis (Micro) System
- Electrophoresis Clean-Up
- Concentration Systems
- Contamination Removal

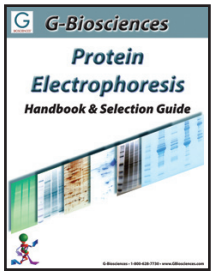




- Protease Inhibitor Cocktails
- Individual Protease Inhibitors
- Protease Assays
- Proteases for Mass Spec.
- Sequencing Grade Proteases

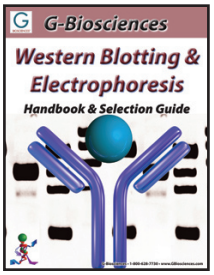
- Proteomic Grade Detergents
- Research Grade Detergents
- Non-Ionic, Ionic & Zwitterionic
- Detergent Estimations
- Detergent Removal Systems

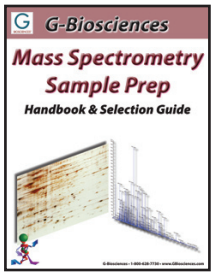




- Gel Preparation Chemicals
- Protein Marker Ladders
- Electrophoresis Buffers
- Reducing & Alkylating Reagents
- Protein Gel Stains

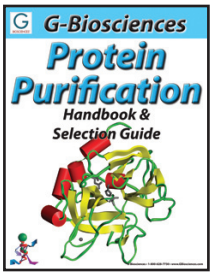
- 1-Hour Western System
- Transfer Buffers & Membranes
- Membrane Stains
- Blocking Buffers
- Secondary Antibodies
- Detection Reagents
- Reprobing Reagents

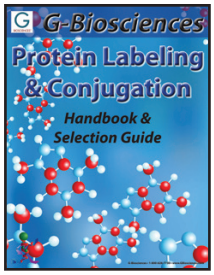




- Protein Sample Preparation
- Protein Clean-Up Systems
- Electrophoresis Reagents
- Mass Spec Grade Protease
- InGel Digestion Kits
- Peptide Generation Reagents

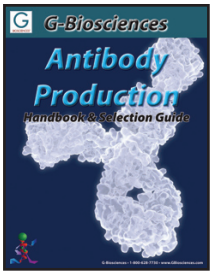
- Affinity Resins
- 6X His Protein Purification Kits
- GST Protein Purification Kits
- Antibody Purification
- Activated Resins
- Buffers & Reagents

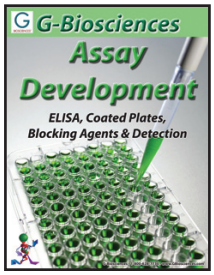




- Biotin Labeling
- Cell Surface Protein Labeling
- Agarose Coupling Kits
- Fluorescent Dye Labeling Kits
- Enzyme Labeling Systems

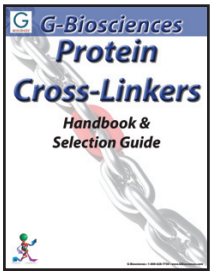
- Carrier Proteins
- Peptide Coupling Systems
- Antibody Purification Resins
- Antibody Fragmentation Kits

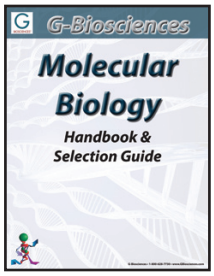




- Coated Plates
- Blocking Buffers
- Wash Buffers
- Secondary Antibodies
- Detection Reagents
- Antibody Labeling Systems

- Homobifunctional
- Heterobifunctional
- Optimizer Systems
- Cross-Linking Systems





- DNA Isolation
- Transformation & Screening
- Polymerase Chain Reaction
- Agarose Electrophoresis
- RNA Isolation
- Yeast Transformation

- Apoptosis Assays
- Cytotoxicity Assays
- SAM Methyltransferase Assays
- Protease Assays
- Phosphatase Assays
- Peroxide Assay
- ELISA

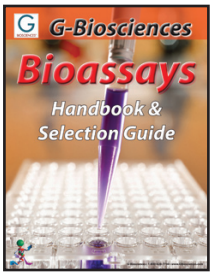


Table of Contents

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 systems 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 proteins into their constituent's polypeptides and hence examine 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 routinely used before Western blotting.

Western blotting is named after a similar technique, Southern blotting, which is the transfer of DNA to a membrane; a technique invented by the British biologist Edwin Southern. Northern blotting is a similar technique, but for RNA. Western blotting is an important technique that is routinely used in research and diagnostic laboratories. Western blotting follows polyacrylamide gel electrophoresis. Western blotting consists of the transfer of the separated proteins onto a membrane where they can be identified with specific antibodies.

The key feature of Western blotting is the use of immunodetection to identify a specific protein, for example a protein marker for a disease. Once the proteins are immobilized on a protein binding membrane, usually nitrocellulose or PVDF (polyvinylidene fluoride), they can be probed with a primary antibody, an antibody specific for the protein of interest. Once bound the antibody is visualized, either with a specific tag coupled to the primary antibody or with a secondary antibody. The secondary antibody is a general antibody that recognizes the constant domain of immunoglobulin G and is species specific. So, if the primary antibody is a mouse antibody, the secondary antibody used will recognize all mouse antibodies. If a secondary antibody is used then this will carry the tag that allows visualization of the protein (see figure).

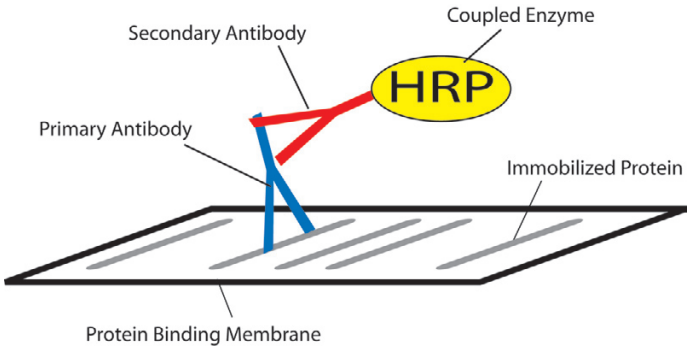


Figure 1: Scheme depicitng the detection of membrane immobilized proteins.

The most common tags used in Western blot are enzymes that catalyze a substrate to produce either light that is detected with radiography film, or color that is visualized on the membrane. The enzymes of choice are horseradish peroxidase (HRP) and alkaline phosphatase (AP). The enzymes are able to catalyze a chemical substrate to produce either a chemiluminescence (light) or colorimetric (color) product that can be detected. This experiment uses HRP and a colorimetric substrate known as 3,3',5,5'-tetramethylbenzidine (TMB).

An additional step is crucial to Western blot and this is known as the blocking step. The blocking step is used to increase the specificity of the Western blot technique by preventing non-specific interactions. If the membranes are not blocked then the antibodies can stick to non-specific proteins due to their charge. To prevent this, the membrane is placed in a protein mixture and the proteins block the charges that would attract the antibodies. Several blocking agents are used, including dried milk powder, bovine serum albumin and casein, however modern blocking agents use synthetic and/or non-animal proteins to prevent any cross reaction with the animal antibodies.

Secondary antibodies recognize and bind to primary antibodies in immunoassays (e.g. Western blots). Secondary antibodies are prepared in the same manner as primary antibodies and the antigen is antibodies from a different species, normally a fragment containing the constant (conserved) domain.

PROTEIN GEL PREPARATION

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. No. 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. No. R014)*	200	320				
10% APS (µl) *(Cat. No. 786-510)*	200	320				
TEMED (µl) *(Cat. No. 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 prepared from electrophoresis grade acrylamide and bis-acrylamide 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. No.	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)

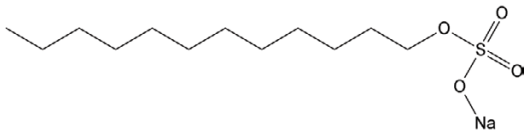


Figure 2: Structure of SDS.

Type: Anionic detergent
Mol. Formula: $C_{12}H_{25}NaO_4S$
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. No.	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: $H_8N_2O_8S_2$
- Molecular Weight: 228.18

Cat. No.	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: $C_6H_{16}N_2$
- Molecular Weight: 116.24

Cat. No.	Description	Size
RC-101	TEMED	50ml
RC-102	TEMED	100ml

PROTEIN MARKERS

PAGEmark™ Protein Ladder

Our prestained PAGE*mark*™ Markers are ~7 to >210kDa* ladder of eight proteins. PAGE*mark*™ proteins are covalently coupled to a blue dye and PAGE*mark*™ 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 PAGE*mark*™ 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*™ PAGE*mark*™ lsingle use format prevents contamination from repeated withdrawals from the same tube.

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

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

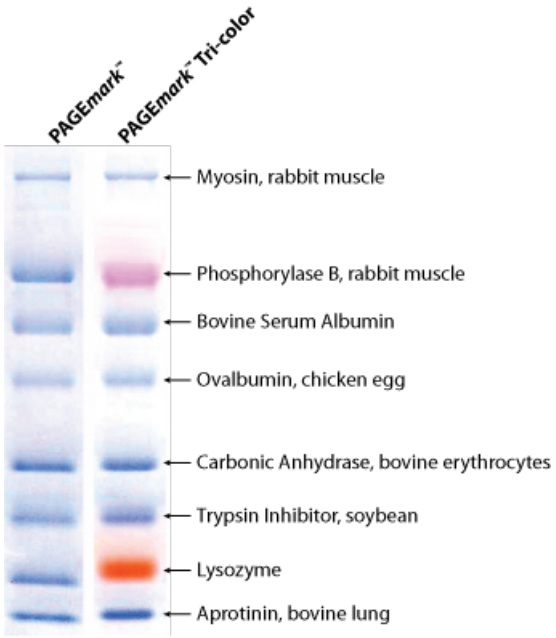


Figure 3: PAGEmark™ and PAGEmark™ Tri-color comparison.

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. No.	Description	Size
786-412	PAGE*mark*™ Tri-color	100 loads
786-415	PAGE*mark*™	100 loads
786-416	*OneQuant*™ PAGE*mark*™	40 loads
786-417	*OneQuant*™ PAGE*mark*™ 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. No.	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. No.	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. No.	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. No.	Description	Size
786-531	MES SDS running buffer [10X]	500ml
786-532	MOPS SDS Running Buffer [20X]	500ml

PROTEIN REDUCTION

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 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

CITED REFERENCES

Jamaluddin, M et al (2010) J. Virol. 84:9533

Cat. No.	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. No.	Description	Size
BC99	DTT	5g
786-077	OneQuant™ DTT [0.5M]	40 vials

ß-Mercaptoethanol

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

Cat. No.	Description	Size
BC98	β-mercaptoethanol	100ml

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

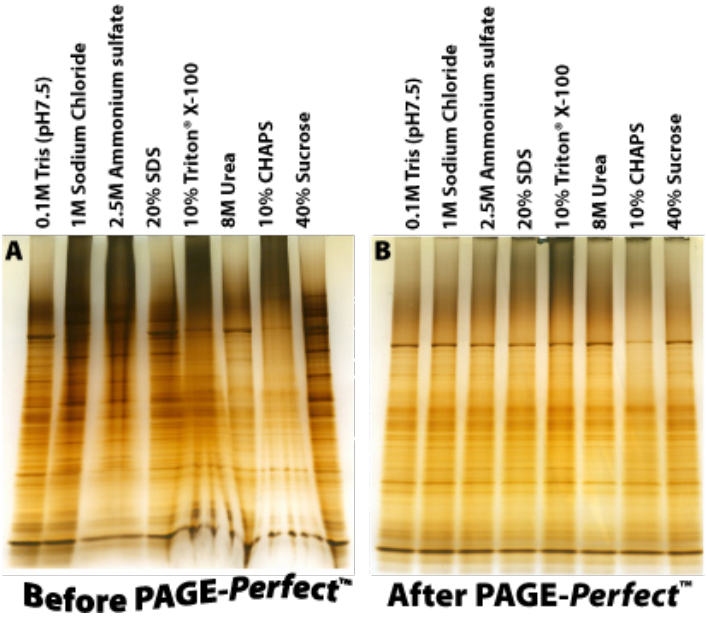


Figure 4: Analysis of mouse liver lysate before and after treatment with PAGE-Perfect™. A. 10µg mouse liver lysates, in the presence of various interfering agents, were loaded onto a SDS polyacrylamide gel. B. 10µg mouse liver lysates, in the presence of various interfering agents, were treated with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

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

RAPID TRANSFER SYSTEM

SWIFT™ Transfer Pads

Enhanced protein transfer, including high molecular weight proteins

Western blot analysis of proteins is a routine and commonly used technique in research laboratories, with 3 major drawbacks. The first is the amount of time taken to transfer the proteins to a protein binding membrane; the second is the variable efficiency of the transfer and the third is problems in transferring high molecular weight proteins. Other minor drawbacks also exist with the Western blotting technique and these include overheating of the apparatus, shorting out of power packs due to excess current and the messy assembling of transfer sandwiches.

SWIFT™ transfer pads alleviate the above issues with Western blotting, when incorporated in the Western blot sandwich. Each SWIFT™ transfer pad can reduce transfer time by up to 50%, while consistently producing high efficiency transfer. The SWIFT™ transfer pad technology prevents overheating and power shortages by allowing lower chemical concentrations in the transfer buffers, without affecting transfer efficiency. The SWIFT™ transfer pad technology combines the simplicity of semi-dry sandwich assembly with the improved efficiency of wet blot transfers, reducing the need for assembly in large tanks of buffer.

SWIFT™ transfer pads are treated with a proprietary electrolyte buffer to enhance Western blot transfer efficiency.

SWIFT™ is compatible with any transfer system, is supplied with or without nitrocellulose or PVDF membranes, and is available in Mini (7.5 x 8.5cm) or Medi (9.5 x 15cm). SWIFT™ Mini is for 10 Western blots and the SWIFT™ Medi is for 5 Western blots.

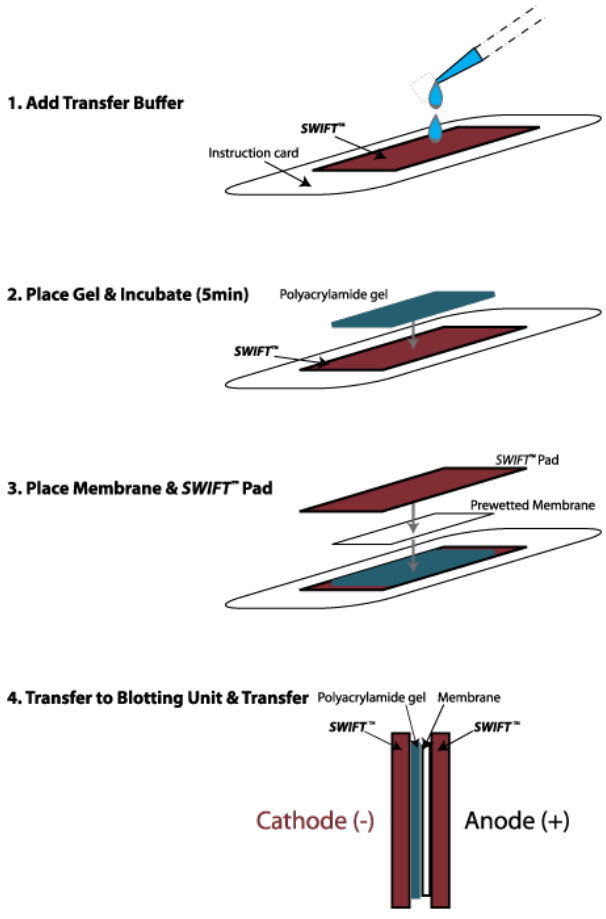


Figure 5: SWIFT™ transfer pad scheme.

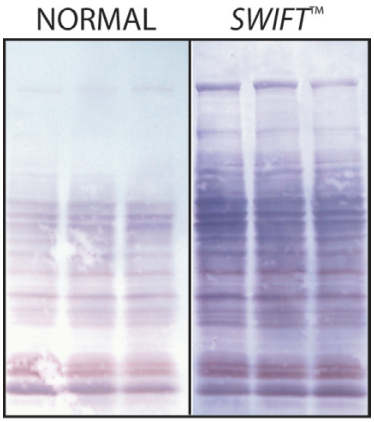


Figure 6: Increased efficiency in protein transfer by SWIFT™ transfer pad. 15µg mouse liver lysate was transferred normally (left) or with a SWIFT™ transfer pad (right) for 30 minutes and the resulting membranes were stained for protein with BLOT-FastStain™.

FEATURES

- High efficiency protein transfer
- Reduce transfer time by up to 50%
- No overheating or power shorts
- No distortion or poor high molecular weight protein transfer

APPLICATIONS

- All Western blot applications
- For improved transfer of high molecular weight proteins

Cat. No.	Description	Size
786-370	*SWIFT*™ Mini transfer pad	10
786-371	*SWIFT*™ Mini transfer pad with nitrocellulose	10
786-372	*SWIFT*™ Mini transfer pad with PVDF	10
786-373	*SWIFT*™ Medi transfer pad	5
786-374	*SWIFT*™ Medi transfer pad with nitrocellulose	5
786-375	*SWIFT*™ Medi transfer pad with PVDF	5

NITROCELLULOSE & PVDF MEMBRANES

Pre-cut transfer membranes and padding for Western blot transfer procedures. Pre-cut membranes are supplied sandwiched between blotting paper padding. Simply soak the membrane in transfer buffer and assemble with the gel in a transfer cassette. Nitrocellulose and PVDF (Polyvinylidene difluoride) membranes are available in 7.5 x 8.5cm or 10 x 10cm sizes.

Cat. No.	Description	Size
786-018NC	Nitrocellulose membrane & padding (7.5 x 8.5cm)	20
786-018PV	PVDF membrane & padding (7.5 x 8.5cm)	20
786-056NC	Nitrocellulose membrane & padding (10 x 10cm)	10
786-056PV	PVDF membrane & padding (10 x 10cm)	10

TRANSFER BUFFERS

Efficient™ Western Transfer Buffer

For increased protein transfer efficiency

A ready-to-use 20X transfer buffer is prepared for optimal conductivity and efficient protein transfer without generating excessive heat or transfer distortion. Efficient™ Western Transfer Buffer achieves greater protein transfer compared to our leading competitors.

FEATURES

- 20X concentrated buffer
- Higher transfer efficiency
- Reduced heat production
- Reduced transfer distortion

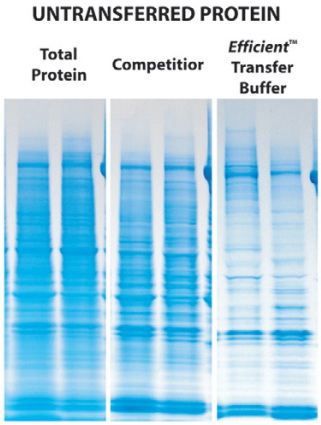


Figure 7: A comparison of protein transfer efficiency between Efficient™ Western Transfer Buffer stained gel after transfer (lanes 5-6) and a leading competitor stained gel after transfer (lanes 3-4). The total protein, before transfer, is shown in lanes 1 and 2. The image show the untransferred protein remaining in the gels. A high concentration of protein (75µg) was loaded to clearly show the difference in the transfer buffers.

Cat. No.	Description	Size
786-019	*Efficient*™ Western Transfer Buffer [20X]	1L

High Molecular Weight Transfer Buffer [5X]

Based on Efficient™ Western Transfer Buffer, the High Molecular Weight Transfer buffer is designed to facilitate the transfer of notoriously difficult high molecular weight proteins (>70kDa) during Western blotting.

Supplied as 1 liter of a 5X concentrated solution.

CITED REFERENCES

Rhee, S. et al (2009) J. Pharmacol. Exp. Ther. 329: 775 - 782

Cat. No.	Description	Size
786-423	High Molecular Weight Transfer Buffer [5X]	1L

MEMBRANE STAINS

Swift™ Membrane Stain

30 second, reversible & sensitive membrane stain

A unique, proprietary, reversible, ready-to-use membrane stain for proteins on nitrocellulose or PVDF membranes. Swift™ Membrane Stain stains proteins faster and with 500X more sensitivity than the routinely used Ponceau-S stain. The lower detection limit is ~0.5ng protein (BSA)/band on nitrocellulose membrane.

Only stains proteins resulting in a clear background and no requirement for additional steps to remove background. The stronger staining allows for easier image capture due to the strong blue stain on a clear, white background.

Swift Membrane Stain™ can be complete removed from the membrane in <1 minute without affecting the biological or immunological properties of the immobilized proteins. This offers an advantage over Coomassie based stains as these are irreversible and can interfere with Western blotting. Suitable for 20 membranes (8 x 10cm).

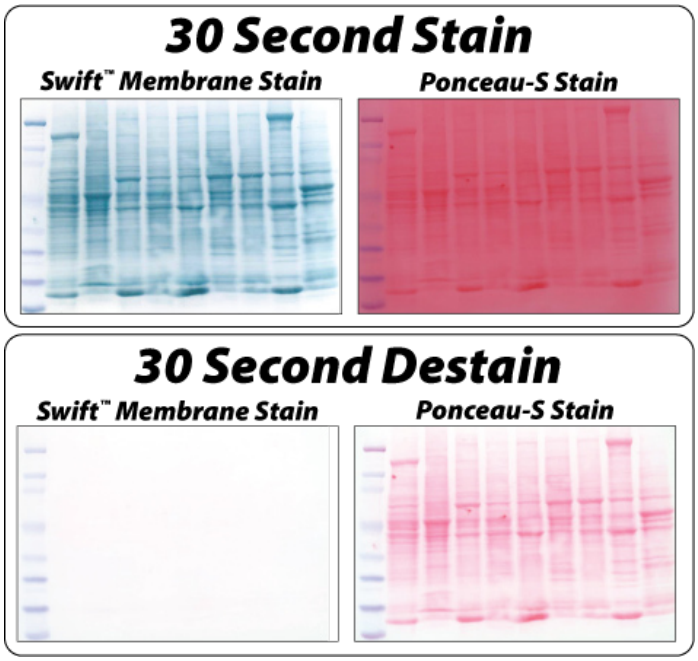


Figure 8: A normal rat multiple tissue blot was probed with Swift™ Membrane Stain or Ponceau-S, using the procedure in "The Protein Protocols Handbook"1. In both instances the membranes were incubate with the respective stain for 30 seconds, rinsed in deionized water and destained as instructed for 30 seconds. Ponceau-S was succesfully destained after >1 hour.

FEATURES

- Reversible stain for protein membranes
- Compatible with nitrocellulose or PVDF
- 500X more sensitive than Ponceau-S (~0.5ng vs. 100ng BSA)
- Outperforms routinely used Ponceau-S

APPLICATIONS

- For visualization of proteins on membranes after Western transfer and dot-blot applications
- Offers simpler image capture

REFERENCES

Kruger, N.J. (1996) Detection of Polypeptides on Blots Using Secondary Antibodies or Protein A. In J. M. Walker (Ed.), The Protein Protocols Handbook (pp. 313-321). New Jersey: Humana Press

Cat. No.	Description	Size
786-677	*SWIFT*™ Membrane Stain	20 blots

BLOT-FastStain™

Sensitive and reversible protein membrane stain

A unique stain for reversible staining of protein on nitrocellulose and PVDF transfer membranes.

BLOT-FastStain™ only stains protein and leaves the background absolutely untouched and brilliant white resulting in high band visibility. The staining procedure takes 10 minutes and has a sensitivity of 2ng BSA, higher than silver stains. Destain the membrane by simply rinsing in warm water for 10 minutes.

Staining and destaining does not affect the biological properties of the proteins. After destaining, protein bands can be probed with Western blot protocols and other analyses including sequencing work.

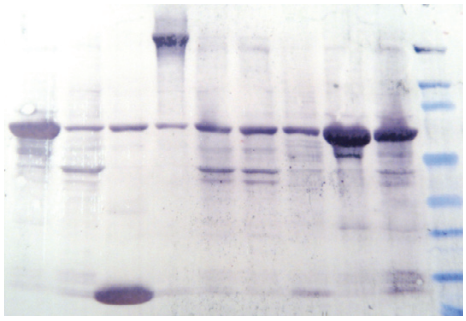


Figure 9: A PVDF membrane stained with BLOT-FastStain™.

FEATURES

- Reversible stain for protein membranes
- Compatible with PVDF and nitrocellulose membranes
- Detect >2ng protein

APPLICATIONS

- For visualization of proteins on membranes after Western transfer and dot-blot applications

CITED REFERENCES

Sarasin-Filipowicz, M. et al (2009) Mol. Cell. Biol. 29: 4841
McDonagh, B. and Sheehan, D. (2006) Aquatic Toxicology. 79: 325
Zagranichnaya, T.K. et al (2005) Physiol. Genomics 21:14
Duong, F.H. et al (2004) J. Virology 79: 15342
Duong, F.H. et al (2004) Gastroenterology 126: 263
Kang, J. and Turano, F.J. (2003) PNAS 100: 6872
Mizgerd, J. et al (2002) Am. J. Respir. Cell Mol. Biol. 27: 575
Aksenov, M. et al (2002) J Neurochem. 74: 6: 2520
Dautzenberg, F. et al (2001) Am. J. Physiol Regul. Integr. Comp. Physiol. 280: R935
Tsibris, J.C.M. et al (1999) Cancer Res. 59: 5737
Yamashita, H. et al (1999) Mol. Human Reproduction 5: 358

Cat. No.	Description	Size
786-34	BLOT-*FastStain*™	25 Blots

Ponceau-S Stain

Ready-to-use 0.1% Ponceau S solution for staining PVDF and nitrocellulose membranes.

Cat. No.	Description	Size
786-575	Ponceau-S stain [0.1%]	250ml
786-576	Ponceau-S stain [0.1%]	500ml

NON-ANIMAL BLOCKING AGENTS

A major drawback of animal protein blocking solutions, such as BSA, casein and milk powders, is they are derived from animal sources. The presence of animal proteins can often lead to high non-specific backgrounds as antigens and antibodies, generated in animals, interact with the "blocking" animal proteins.

NAP-BLOCKER™

Non-animal blocking protein preparation

For improved assay sensitivity, minimal non-specific binding, and a high signal-to-background ratio. NAP-BLOCKER™ ensures no cross-reaction with your animal source antigens and antibodies, due to being 100% free of animal proteins. NAP-BLOCKER™ is easy to use and generates high publication quality blots.

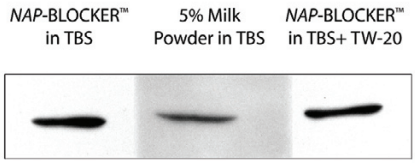


Figure 10: Comparison of NAP-BLOCKER™ and milk powder. Protein lysates were transferred to PVDF membranes and blocked for 90 minutes as indicated. The membranes were probed for actin and subsequently exposed to film for 20 minutes.

NAP-BLOCKER™ is free from biotin and other cross-reacting agents present in most of the animal source blocking agents. NAP-BLOCKER™ ensures uniform blocking without non-specific binding. It is simple to use with improved results compared to milk powder preparations.

NAP-BLOCKER™ is supplied as a pre-made [2X] solution; simply dilute with any buffer and block nitrocellulose or PVDF membranes. Alternatively, NAP-BLOCKER™ is supplied in PBS or TBS buffers.

FEATURES

- Non-animal protein blocking agent
- 2X concentrated solution
- Uniform blocking with reduced background staining

APPLICATIONS

- For Western blots, dot blots, ELISA and assay development

CITED REFERENCES

Reynolds, J. L. et al (2012) J. Immunol. 188:3757
Subramaniam, R. et al (2011) Clin. Vaccine Immunol. 18:1689
Walsh, R. and Camilli, A. (2011) mBio. 2:e00092-11
McGrath, M., et al (2008) J. Agric. Food Chem. 56: 7044-48.
Yamaza, T. et al (2008) PLoS ONE 3(7): e2615. doi:10.1371/journal.pone.0002615
Ruscheinsky, M. et al (2008) Matrix Bio. 27: 487-497
Maruscak, A., et al (2008) Am. J. Physiol. Lung Cell Mol. 294:L974
Tornetta, M., et al (2007) J. Immunol. Methods. 328: 34-44.
Crook, J.D., et al (2007) Neuroscience. 149(4): 834-44.
Courter, L.A. et al (2007) Tox. Sci. 95:63.
Hui, L. et al (2006) Biol. Reproduction. 74:633
Mahadavan, B. et al (2005) Cancer Res. 65: 1251
Musafia-Jeknic, T. et al (2005) Tox Sci 88: 358
Shulby, S. et al (2004) Cancer Res. 64: 4693
Qin, M. et al (2003) Clin. Cancer Res. 9: 4992
Rice, D. et al (2002) Hypertension. 39: 502
Wesselman, J. et al (2001) Hypertension. 37: 955
Chrysis, D. et al (2001) J. Neurosci. 21: 1481
Thomas, R. et al (2000) Clin. Cancer Res. 6: 1140
Ginkel, L. et al (2000) Mol. Biol. Cell. 11: 4143

Cat. No.	Description	Size
786-190	NAP-BLOCKER™ [2X]	2 x 500ml
786-190P	NAP-BLOCKER™ in PBS [2X]	2 x 500ml
786-190T	NAP-BLOCKER™ in TBS [2X]	2 x 500ml

Protein-Free™

Eliminates protein related cross-reactivity

Protein-Free Blocking Buffer does not contain protein; it is a proprietary formulation of non-protein agents that eliminates non-specific binding sites in ELISA, blotting, immunohistochemistry and other applications. The absence of protein eliminates problems associated with traditional protein based blockers, such as cross-reactivity and interference from glycosylated proteins.

Eliminates any concern associated with regulatory compliance issues where use of animal source components are restricted. Furthermore, Protein-Free™ Blocking Buffer is compatible with antibodies and avidin/biotin based systems and results in high signal to background ratios.

For user's convenience Protein-Free Blocking Buffers are supplied in widely used TBS (Tris-buffered saline at pH 7.5) and PBS buffers (phosphate-buffered saline at pH 7.5) as well as in separate formulations containing Tween® 20 for improving blocking efficiencies.

FEATURES

- Protein free blocking agent
- Eliminate cross reactivity with animal source antibodies
- High signal to background ratios
- Four convenient formats, with and without detergent
- Ready-to-use

APPLICATIONS

- Suitable for Western blot and ELISA applications

Cat. No.	Description	Size
786-664	Protein-Free Blocking Buffer-PBS	500ml
786-665	Protein-Free Blocking Buffer-PBST	500ml
786-662	Protein-Free Blocking Buffer-TBS	500ml
786-663	Protein-Free Blocking Buffer-TBST	500ml

NON-ANIMAL SERA PROTEIN BLOCKING AGENTS

FISH-Blocker™

Uses fish proteins to eliminate cross reactivity

FISH-Blocker™ is a blocking agent that uses a fish protein as the primary blocking agent. The use of a fish protein, a non-mammalian protein, is that it eliminates or minimizes the interaction of antibodies raised in mammals. FISH-Blocker™ is one of the best blocking agents for immunoassays and it offers an alternative to milk-based blocking agents, minimizing the risk of non-specific binding of antibodies during the immunodetection process and lowering the background.

FEATURES

- A non mammailan protein to elimate non-specific binding
- High signal to background ratio
- Ready-to-use

APPLICATIONS

- Suitable for Western blot and ELISA applications

Cat. No.	Description	Size
786-675	FISH-Blocker™ in PBS	500ml
786-674	FISH-Blocker™ in TBS	500ml

Superior™ Blocking Buffer

An enhanced blocker in multiple formats

Superior™ Blocking Buffer contains a proprietary antigenically non-determinant protein for blocking non-specific sites during ELISA, membrane blotting, immunohistochemistry and other applications.

Superior™ Blocking Buffer is ideal for a high signal to background ratio in most system. Superior™ Blocking Buffer uses a non-serum protein and does not contain biotin or other animal source proteins to interfere with immuno-complexes. Superior™ Blocking Buffer is suitable for assays that use avidin/streptavidin systems.

Superior™ Blocking Buffer for Precipitating Substrate is a modification of Superior™ Blocking Buffer. This blotting buffer has been optimized for use in blotting protocols that use precipitating substrates, such as our femtoCHROMO™ chromogenic detection systems, TMB (3, 3', 5, 5'-Tetramethylbenzidine), BCIP (5-Bromo-4-Chloro-3'-Indolyphosphate p-Toluidine Salt) and NBT (Nitro-Blue Tetrazolium Chloride) substrates. Superior™ Blocking Buffer for Precipitating Substrate is not suitable for ELISA or immunohistochemistry staining.

Available in multiple formats using TBS, PBS, TBS with 0.05% Tween® 20 or PBS with 0.05% Tween® 20. Also supplied as a convenient dry form that is stable at room temperature. Each dry format pack makes 200ml Superior™ Blocking Buffer.

FEATURES

- Non serum protein blocking agent
- Rapid blocking times; ~2 minutes for ELISA
- Multiple formats; ready-to-use liquid (500ml) or dry buffer packs (for 5 x 200ml)
- Available with and without detergent (Tween® 20)

APPLICATIONS

- For blocking Western blot membranes (PVDF and nitrocellulose)
- For blocking and storage of ELISA plates
- For blocking prior to immunohistochemistry staining

Superior™ Blocking Buffer for Precipitating Substrate is not suitable for ELISA or immunohistochemistry staining.

Cat. No.	Description	Size
786-660	Superior™ Blocking Buffer in PBS	500ml
786-661	Superior™ Blocking Buffer in PBST	500ml
786-658	Superior™ Blocking Buffer in TBS	500ml
786-659	Superior™ Blocking Buffer in TBST	500ml
786-601	Superior™ Blocking Buffer-Dry Blend in PBS	5 packs
786-657	Superior™ Blocking Buffer-Dry Blend in TBS	5 packs
786-656	Superior™ Blocking Buffer for Precipitating Substrate in PBS	500ml
786-655	Superior™ Blocking Buffer for Precipitating Substrate in TBS	500ml

FirstChoice™

Ideal for new assay development

A proprietary protein formulation that offers greater versatility and lack of cross-reactivity. FirstChoice™ Blocking Buffer is ideal as a first choice for optimization of new assays, systems or when determining the optimal blocking buffer for elimination of non-specific binding sites in ELISA, blotting, immunohistochemistry and other applications. FirstChoice™ Blocking Buffers are compatible with antibodies and avidin/biotin based systems and results in high signal to background ratios.

For users convenience FirstChoice™ Blocking Buffers are supplied in widely used TBS (Tris-buffered saline at pH 7.5) and PBS (phosphate-buffered saline at pH 7.5) buffers as well as in separate formulations containing Tween® 20 for improving blocking efficiencies.

FEATURES

- Ready-to-use
- For Western blotting and ELISA
- Available as TBS or PBS with optional Tween® 20
- Animal serum free
- Biotin free

APPLICATIONS

- Ideal blocking buffer for setting up new assays and systems

Cat. No.	Description	Size
786-668	FirstChoice™ Blocking Buffer-PBS	500ml
786-669	FirstChoice™ Blocking Buffer-PBST	500ml
786-666	FirstChoice™ Blocking Buffer-TBS	500ml
786-667	FirstChoice™ Blocking Buffer-TBST	500ml

BLOT-QuickBlocker™

A modified milk protein blocking agent

BLOT-QuickBlocker™ is a novel modified milk protein that is highly soluble and does not inhibit peroxidase detection. The modified milk protein has high blocking efficiency with a clear background.

FEATURES

- Readily soluble and produces semi-clear solution
- No inhibition to peroxidase
- Produces clear background
- Higher blocking efficiency
- Blocking time 30-60 minutes
- Fat free

APPLICATIONS

- For Western blots and dot blots

CITED REFERENCES

Sow, F. et al (2009) J. Leukoc. Biol. 86: 1247
Malloy, P. and Feldman, D. (2009) Endocrinology 150: 679
Kroemer, J.A and Webb, B.A. (2006) J Virol. 80: 12219
Benou, C. et al (2005) J. Immunology. 174: 5407
Wang, Y. et al (2005) J. Immunol. 174: 5687
Kroemer, J.A and Webb, B.A. (2005) J Virol. 79: 7617
Bakke, L.J. et al (2004) Biol. Reprod. 71:605
Li, Q. et al (2004) Reproduction 128: 555
Alvarez, G. R. et al (2003) J. Immunol. 171: 6766

Cat. No.	Description	Size
786-011	BLOT-QuickBlocker™	175g

BLOK™ BLOTTO

A 5% modified milk protein blocking solution

FEATURES

- 5% novel modified milk protein solution
- Use for Westerns, ELISA, and dot blot blocking
- Blocking in 10-15 minutes
- Ready-to-use

Cat. No.	Description	Size
786-192	BLOK™ BLOTTO 5% non fat milk solution	2 x 500ml

BLOK™ Casein

A 1% casein protein blocking solution

FEATURES

- Ready-to-use
- Available in your choice of TBS or PBS buffers.

Cat. No.	Description	Size
786-194	BLOK™ Casein in PBS, 1% solution	2 x 500ml
786-196	BLOK™ Casein in TBS, 1% solution	2 x 500ml

PROTEIN BLOCKING AGENTS

BLOK™ BSA

A 10% BSA protein blocking solution

- For blocking Westerns, ELISA and dot Blots
- Ready-to-use
- Available in your choice of TBS or PBS buffers

Cat. No.	Description	Size
786-193	BLOK™ BSA in TBS, 10% solution	125ml
786-195	BLOK™ BSA in PBS, 10% solution	125ml

PHOSPHATE BUFFER SALINE (PBS)

femtoPBST™ Wash Buffer

10X concentrated PBS wash buffers to enhance sensitivity of your immunoassays by minimizing "washing out" of antibodies.

CITED REFERENCES

Hui, L. et al (2005) Biol. Reproduction 10: 1095
Li, Q. et al (2004) Reproduction 128:555
Bakke, L.J. et al (2004) Biol. Reprod. 71:605
Calhoun, D.H. et al (2001) Genome Biol. 2:1

Cat. No.	Description	Size
786-162	*femto* PBST™ [10X]	250ml

10X PBS

10X concentrated PBS solutions for the use as wash buffers for Western blotting, ELISA and other applications.

Cat. No.	Description	Size
786-027	PBS [10X]	500ml
R027	PBS [10X]	1L
R028	PBS [10X]	1gal

Dry Buffer Packs

Just add water to generate ready-to-use buffers

- **JAW™ Phosphate Buffered Saline (PBS) [1X]** packs make 1L of 2.7mM potassium chloride, 127mM sodium chloride and 10mM phosphate buffer (pH 7.3-7.5)
- **JAW™ Phosphate Buffered Saline (PBS) [10X]** packs make 1L of 27mM potassium chloride, 1.37M sodium chloride and 0.1M phosphate buffer (pH7.3-7.5)

Cat. No.	Description	Size
786-289	JAW™ Phosphate Buffered Saline [1X] (1L/pack)	20 packs
RC-147	JAW™ Phosphate Buffered Saline [10X] (10L/pack)	2 packs

TRIS BUFFERED SALINE (TBS)

femtoTBST™ Wash Buffer

10X concentrated TBS wash buffers to enhance sensitivity of your immunoassays by minimizing "washing out" of antibodies.

CITED REFERENCES

Hui, L. et al (2005) Biol. Reproduction 10: 1095
Li, Q. et al (2004) Reproduction 128:555
Bakke, L.J. et al (2004) Biol. Reprod. 71:605
Calhoun, D.H. et al (2001) Genome Biol. 2:1

Cat. No.	Description	Size
786-161	*femto* TBST™ [10X]	250ml

10X TBS

10X concentrated TBS solutions for the use as wash buffers for Western blotting, ELISA and other applications.

Cat. No.	Description	Size
R029	TBS [10X])	1L
R030	TBS [10X])	1gal

Dry Buffer Packs

Just add water to generate ready-to-use buffers

- **JAW™ Tris Buffered Saline [1X]** packs make 1L of 25mM Tris, 140mM sodium chloride, 3mM potassium chloride, pH 7.25-7.55
- **JAW™ Tris Buffered Saline (TBS) [20X]** packs make 1L of 0.5M Tris, 2.8M sodium chloride, 60mM potassium chloride, pH 7.25-7.55

Cat. No.	Description	Size
786-288	JAW™ Tris Buffered Saline [1X] (1L/pack)	20 packs
RC-148	JAW™ Tris Buffered Saline [20X] (20L/pack)	1 pack

TWEEN® 20, PROTEOMIC GRADE

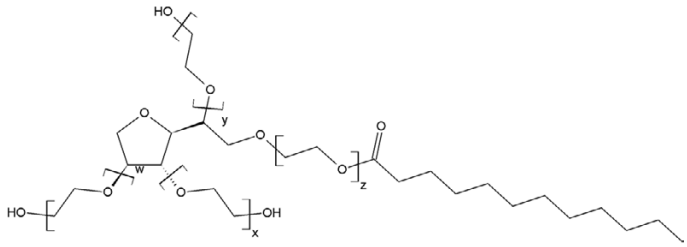


Figure 11: Structure of Tween® 20.

Contains reduced peroxides and carbonyl compounds. In addition, the detergent has less than 50µS conductivity. The proteomic grade Tween® 20 detergent is offered as 10% aqueous solutions, sealed under inert gas and are suitable for protein applications.

Tween® 20 is routinely used as an additive to membrane wash buffers to enhance washing steps, resulting in a clearer background.

FEATURES

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

Type: Non-ionic detergent
Form: 10% aqueous solution (w/v)
Mol. Formula: $C_{18}H_{34}O_6 \cdot [C_2H_4O]_{w+x+y+z}$ for w+x+y+z =20
Mol Weight: ~1227 (for w+x+y+z =20)
Absorbance (215nm): 0.05 (0.05% w/v)
Aldehyde content: < 100µM
Peroxide content (as H_2O_2): < 50µM
Critical micelle concentration (CMC): approx 0.06 x 10^{-3}M
Cloud Point: 76°C

Cat. No.	Description	Size
DG011	Tween® 20, 10% solution	5 x 10 ml vials
DG012	Tween® 20, 10% solution	10 x 10 ml vials
DG511	Tween® 20, 10% solution	50 ml bottle
DG519	Tween® 20, 10% solution	100 ml bottle

HORSERADISH PEROXIDASE (HRP) CONJUGATED

Affinity purified Horseradish peroxidase (HRP), for conjugation to a labeled molecule, produces a colored, fluorimetric or luminescent derivative of the labeled molecule, allowing it to be detected and quantified. HRP is ideal for secondary antibody conjugation because it is smaller, more stable and less expensive than other popular alternatives. It also has a high turnover rate that allows generation of strong signals in a relatively short time span. The activity of the HRP enzyme is inhibited by cyanides, azides and sulfides.

The antibodies are isolated from antisera by immunoaffinity chromatography using antigen coupled to sepharose beads.

- Supplied lyophilized from 0.01M sodium phosphate, 0.25M NaCl, pH7.1 with 15mg/ml BSA and 0.01% thimerosal
- Western blotting/Immunoblotting: 1:5,000-1:100,000
- ELISA: 1:5,000-1:100,000
- Immunohistochemistry: 1:500-1:5,000

CITED REFERENCES

Van Zandt, K. et al (2008) Biology 689-700
Polkinghorne, A. et al (2008) Microbiology 154: 3537-46
Li, Q. et al (2006) Reproduction 131:533
Benou, C. et al (2005) J. Immunology. 174: 5407
Wang, Y. et al (2005) J. Immunol. 174: 5687
Bakke, L.J. et al (2004) Biol. Reprod. 71:605
Li, Q. et al (2004) Reproduction 128: 555
Alvarez, Gail R. et al (2003) J. Immunol. 171: 6766

Cat. No.	Description	Size
786-R41	Horseradish peroxidase (HRP) labeled goat α-human IgG	2ml
786-R38	HRP labeled goat α-mouse IgG	2ml
786-R39	HRP labeled goat α-rabbit IgG	2ml
786-R40	HRP labeled goat α-rat IgG	2ml
786-R42	HRP labeled rabbit α-goat IgG	1.5ml
786-R48	HRP labeled rabbit α-human IgG	1.5ml

ALKALINE PHOSPHATASE (AP) CONJUGATED

Alkaline phosphatase (AP) is a large 140kDa protein that hydrolyzes phosphate groups from substrates, resulting in a colored, fluorimetric or luminescent derivative. The antibodies are isolated from antisera by immunoaffinity chromatography using antigen coupled to sepharose beads.

- Supplied lyophilized from 0.01M sodium phosphate, 0.25M NaCl, pH7.1 with 15mg/ml BSA and 0.01% thimerosal
- Western blotting/Immunoblotting: 1:5,000-1:100,000
- ELISA: 1:5,000-1:100,000
- Immunohistochemistry: 1:500-1:5,000

CITED REFERENCES

Van Zandt, K. et al (2008) Biology 689-700
Polkinghorne, A. et al (2008) Microbiology 154: 3537-46
Li, Q. et al (2006) Reproduction 131:533
Benou, C. et al (2005) J. Immunology. 174: 5407
Wang, Y. et al (2005) J. Immunol. 174: 5687
Bakke, L.J. et al (2004) Biol. Reprod. 71:605
Li, Q. et al (2004) Reproduction 128: 555
Alvarez, Gail R. et al (2003) J. Immunol. 171: 6766

Cat. No.	Description	Size
786-R46	Alkaline phosphatase (AP) labeled goat α-human IgG	1ml
786-R43	AP labeled goat α-mouse IgG	1ml
786-R44	AP labeled goat α-rabbit IgG	1ml
786-R45	AP labeled goat α-rat IgG	1ml
786-R47	AP labeled rabbit α-goat IgG	1ml
786-R49	AP labeled rabbit α-human IgG	1ml

ENZYME LABELING KITS

For the rapid and stable coupling of HRP and AP enzymes to proteins

The HOOK™ Enzyme Labeling Kits are designed for the coupling of horseradish peroxidase (HRP) and alkaline phosphatase (AP) to proteins, particularly antibodies.

G-Biosciences offers three enzyme labeling kits that are supplied with all the reagents required for high efficiency enzyme coupling.

HOOK™ HRP PLUS Labeling

A high efficiency enzyme labeling kit for tagging proteins with horseradish peroxidase enzyme. This kit has an activated HRP that couples with high efficiency (>90%) to the numerous amine groups of proteins and is superior to glutaraldehyde coupling chemistry.

Uses HOOK™ HRP PLUS, which is HRP that has been activated by the addition of reactive aldehydes. The aldehyde groups react spontaneously and at high efficiency with primary amines, located at the N-terminus of proteins or in lysine residues, to form intermediate Schiff Base complexes. These, in turn, are selectively reduced by the supplied reduction agent. Following quenching of the reaction the protein is linked to the horseradish peroxidase enzyme by stable amine linkage. The labeled protein, or antibody, can now be used for immunoblotting, ELISA and histochemical techniques.

FEATURES

- Activity is 120-200 units/mg
- Reacts with primary amines to form covalent amine bonds

Cat. No.	Description	Size
786-313	HOOK™ HRP PLUS labeling kit	5 reactions

HOOK™ HRP Sulfo Labeling

An efficient enzyme labeling kit for tagging proteins with horseradish peroxidase (HRP) enzyme. This kit has activated HRP that couples to peptides, proteins and ligands that have free sulfhydryl groups. The maleimide activated HRP saves time as the first step of the normal two-step maleimide activation procedure is already complete, saving several hours of valuable research time.

To aid in the preparation of HRP conjugates using free sulfhydryls the kit is supplied with SATA (N-Succinimidyl S-acetylthioacetate), to add free sulfhydryls to existing amine groups, and 2-mercaptoethylamine.HCl, a mild reducing agent for conjugating HRP to immunoglobulin G (IgG) and its fragments.

Cat. No.	Description	Size
786-314	HOOK™ HRP SULFO labeling kit	5 reactions

HOOK™ AP Sulfo Labeling

An efficient enzyme labeling kit for tagging proteins with alkaline phosphatase enzyme. This kit has activated AP that couples to peptides, proteins and ligands that have free sulfhydryl groups. The maleimide activated AP saves time as the first step of the normal two-step maleimide activation procedure is already complete, saving several hours of valuable research time.

To aid in the preparation of AP conjugates using free sulfhydryls the kit is supplied with SATA (N-Succinimidyl S-acetylthioacetate), to add free sulfhydryls to existing amine groups, and 2-mercaptoethylamine.HCl, a mild reducing agent for conjugating AP to immunoglobulin G (IgG) and its fragments.

Cat. No.	Description	Size
786-315	HOOK™ AP SULFO labeling kit	5 reactions

femtoLUCENT™ PLUS

Highly sensitive detection system

A femtogram level sensitive immunodetection system allows users the option to detect even hard-to-detect low abundance proteins. This immunodetection system is based on an innovative formulation of luminol and 1,2 dioxetane supersensitive detection reagent and a unique, proprietary combination of blocking agent (NAP-BLOCKER™) and washing buffers to ensure low background and high signal to background ratio. Optional horseradish peroxidase (HRP) or alkaline phosphatase (AP) conjugated secondary antibodies against mouse, rabbit, human, rat and goat are also offered for a complete chemiluminescence detection system.

femtoLUCENT™ PLUS is offered as an immunodetection system with all of the critical reagents needed for Western blots or dot-blot analysis or as a reagent kit for those who prefer to customize their detection methods with their own secondary antibody conjugates.

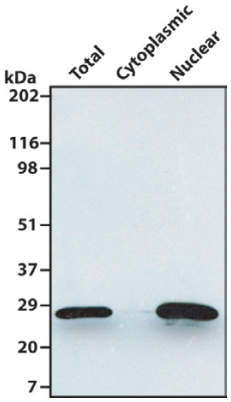


Figure 12: NIH3T3 cells were fractionated with FOCUS™ Cytoplasmic & Nuclear Extraction kit. The fractions were resolved and blotted. The blot was probed with α-caveolin and the protein visualized with femtoLUCENT™ PLUS system.

FEMTOLUCENT™ PLUS KIT INCLUDES

- Detection reagents for HRP or AP
- NAP-BLOCKER™, a non animal protein blocking agent
- femto-TBST™ washing buffer
- Optional secondary antibodies

FEATURES

All of the critical reagents provide

- Economical: Greater value compared to similar products
- Intense light emission with low background : high signal ratio
- Low femtogram detection (10^{-15}), allows detection of >10fg protein on a dot blot and >1pg on a Western blot
- Supplied with a novel blocking agent which allows a rapid blocking step and produces a clear background
- Suitable for nitrocellulose & PVDF membranes

APPLICATIONS

- For Western blots and dot blot applications

CITED REFERENCES

Kenedy, M. and Akins, D. (2011) Infect. Immun. 79:1451
Chen, L. et al (2010) J. Gen. Virol. 91: 382
Stefanini, L. et al (2009) Blood. 114: 2506
Sow, F. et al (2009) J. Leukoc. Biol. 86: 1247
Kenedy, M. et al (2009) Infect. Immun.77: 2773
Van Zandt, K. et al (2008) J. Leukoc. Biol. 84: 689
Fenton, J. et al (2006) Carcinogenesis 27: 1507
Gopalakrishnan, R. and Chandra, N.C. (2006) Ind. J. Clin. Biochem. 21: 8
Wang, Y. et al (2005) J. Immunol. 174: 5687
Li, Q. et al (2004) Reproduction 128:555
Bakke, L.J. et al (2004) Biol. Reprod. 71:605
Li, L. et al (2003) J Biol. Chem. 278: 4725
Alvarez, G. et al (2003) J. Immunology. 171: 6766
Fenton, J. et al (2005) Cancer Epid. 14: 1646
Calhoun, D. et al (2001) Genome Biology. 2(8): research0030.1
Gosset, G. et al (2001) J. Bacteriology. 183: 4061

Cat. No.	Size (For cm^2)	Enzyme Conjugated Secondary Antibodies	NAP-BLOCKER™ & femtoTBST™ Wash Buffer
femtoLUCENT™ PLUS for Horseradish Peroxidase (HRP)			
786-003	1,500	-	-
786-10	1,500	-	Yes
786-10T	300	-	Yes
786-10-R41	1,500	HRP-goat α-human antibody	Yes
786-10-R38	1,500	HRP-goat α-mouse antibody	Yes
786-10-R39	1,500	HRP-goat α-rabbit antibody	Yes
786-10-R40	1,500	HRP-goat α-rat antibody	Yes
786-10-R48	1,500	HRP-rabbit α-human antibody	Yes
786-10-R42	1,500	HRP-rabbit α-goat antibody	Yes
femtoLUCENT™ PLUS for Alkaline Phosphatase (AP)			
786-10AP	1,500	-	Yes
786-10APT	300	-	Yes
786-10AP-R46	1,500	AP-goat α-human antibody	Yes
786-10AP-R49	1,500	AP-rabbit α-human antibody	Yes
786-10AP-R43	1,500	AP-goat α-mouse antibody	Yes
786-10AP-R44	1,500	AP-goat α-rabbit antibody	Yes
786-10AP-R45	1,500	AP-goat α-rat antibody	Yes
786-10AP-R47	1,500	AP-rabbit α-goat antibody	Yes

picoLUCENT™ PLUS

Superior detection with low background

Based on our sensitive luminol and 1,2 dioxetane substrate that produces chemiluminescence upon reaction with horseradish peroxidase or alkaline phosphatase.

Detects low picogram levels (10^{-12}) of antigens with low noise (signal/background) ratio. The kit reagents are sufficient for 1,500cm^2 of PVDF or nitrocellulose membrane.

FEATURES

- Low picogram detection (10^{-12})
- Low background : High signal ratio
- Nitrocellulose or PVDF compatible
- Supplied with blocking and washing reagents

Cat. No.	Size (For cm^2)	Enzyme Conjugated Secondary Antibodies	NAP-BLOCKER™ & femtoTBST™ Wash Buffer
picoLUCENT™ PLUS for Horseradish Peroxidase (HRP)			
786-002	1,500	-	-
786-09	1,500	-	Yes
786-09T	300	-	Yes
786-09-R41	1,500	HRP-goat α-human antibody	Yes
786-09-R38	1,500	HRP-goat α-mouse antibody	Yes
786-09-R39	1,500	HRP-goat α-rabbit antibody	Yes
786-09-R40	1,500	HRP-goat α-rat antibody	Yes
786-09-R48	1,500	HRP-rabbit α-human antibody	Yes
786-09-R42	1,500	HRP-rabbit α-goat antibody	Yes
picoLUCENT™ PLUS for Alkaline Phosphatase (AP)			
786-09AP	1,500	-	Yes
786-09APT	200	-	Yes
786-09AP-R46	1,500	AP-goat α-human antibody	Yes
786-09AP-R49	1,500	AP-rabbit α-human antibody	Yes
786-09AP-R43	1,500	AP-goat α-mouse antibody	Yes
786-09AP-R44	1,500	AP-goat α-rabbit antibody	Yes
786-09AP-R45	1,500	AP-goat α-rat antibody	Yes
786-09AP-R47	1,500	AP-rabbit α-goat antibody	Yes

RAPID BLOT DETECTION SYSTEM

SWIFT™ Western Diluent

Unique, Rapid Development of Western blots

SWIFT™ Western Diluent is a new generation Western blotting reagent. The single reagent SWIFT™ Western Diluent simplifies protein detection by Western blotting and reduces the overall time spent on Western blot development. Traditional Western blotting requires a blocking step to eliminate non-specific binding and the majority of published protocols recommend incubating the blot membrane in blocking solutions from 1hr to overnight. SWIFT™ Western Diluent has been developed to eliminate the time consuming blocking step (see figure).

The SWIFT™ Western Diluent is a unique solution that elimates the blocking step and can reduce antibody incubations on Western blot membranes. SWIFT™ Western Diluent generates comparable result to traditional Western blotting procedures and other commercial "fast" Western blotting kits (see blots below)

An added advantage is that SWIFT™ Western Diluent is designed to be used with any existing combination of primary and secondary antibodies, unlike other commercial kits that limit researcher's to rabbit or mouse primary antibodies.

For added convenience, the SWIFT™ Western Diluent is supplied in a complete kit to ensure optimal results. The kit includes SWIFT™ Western Diluent, proprietary wash buffers and our highly sensitive femtoLUCENT™ chemiluminescence detection reagent.

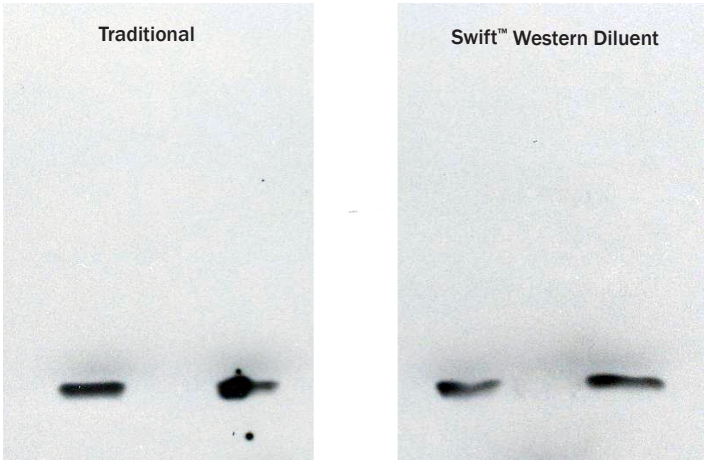


Figure 13: Traditional Western blotting compared to SWIFT™ Western Diluent. Left. Traditional Western blotting method showing the actin protein in liver and lung lysates. Right. SWIFT™ Western Diluent was used to eliminate the blocking step and developed comparable actin protein bands and clean background.

FEATURES

- Affordable: Single reagent
- Fast: Reduce blot development to <90 mins
- Versatile: Compatible with all combinations of primary and secondary antibodies
- For all wet, semi-dry and automated blotting systems

Cat. No.	Description	Size
786-679	SWIFT™ Western Diluent	8 blots
786-158	*SWIFT™* Western Blotting System	8 blots

femtoCHROMO™-AP

Ready-to-use modified BCIP (5-Bromo-4-Chloro-3'-Indolyphosphate p-Toluidine Salt) and NBT (Nitro-Blue Tetrazolium Chloride) substrate that generates a black-purple insoluble precipitate in the presence of alkaline phosphatase.

Supplied with an enhanced blocking agent, BLOT-QuickBlocker™, and a concentrated [10X] washing buffer, femtoTBST™ Buffer to ensure low background staining. Optional AP labeled goat α-mouse or rabbit antibodies are supplied.

FEATURES

- Detects >5ng
- Ready-to-use, single detection step
- High signal to background ratio and reproducibility

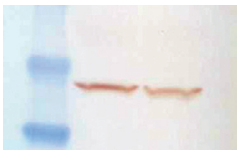


Figure 14: Detection with femtoCHROMO™. Human lysates were transferred to a PVDF membrane, which was probed with actin and alkaline phosphatase labeled goat anti-mouse antibodies. Membrane was probed with femtoCHROMO™-AP substrate.

Cat. No.	Size (For cm²)	Goat AP Conjugated Secondary Antibodies	BLOT-QuickBlocker™ & femtoTBST™ Wash Buffer
femtoCHROMO™-AP for Alkaline Phosphatase (AP)			
786-379	4,000	-	-
786-380	4,000	-	Yes
786-381	4,000	α-mouse antibody	Yes
786-382	4,000	α-rabbit antibody	Yes
786-383	4,000	α-mouse antibody α-rabbit antibody	Yes

femtoCHROMO™-HRP

A ready-to-use modified TMB (3, 3', 5, 5'-Tetramethylbenzidine) substrate is used that generates a dark blue precipitate in the presence of horseradish peroxidase.

Supplied with an enhanced blocking agent, BLOT-QuickBlocker™, and a concentrated [10X] washing buffer, femtoTBST™ Buffer to ensure low background staining. Optional HRP labeled goat α-mouse or rabbit antibodies are supplied.

FEATURES

- Detects >20ng
- High signal to background ratio and reproducibility

Figure 15: Detection with femtoCHROMO™. Human lysates were transferred to a PVDF membrane, which was probed with actin and horseradish peroxidase labeled goat anti-mouse antibodies.Membrane was probed femtoCHROMO™-HRP substrate.

CITED REFERENCES

Bettegowda, A. et al (2007) PNAS 104: 17602

Cat. No.	Size (for cm²)	Goat HRP Conjugated Secondary Antibodies	BLOT-QuickBlocker™ & femtoTBST™ Wash Buffer
femtoCHROMO™-HRP for Horseradish peroxidase (HRP)			
786-384	4,000	-	-
786-385	4,000	-	Yes
786-386	4,000	α-mouse antibody	Yes
786-387	4,000	α-rabbit antibody	Yes
786-388	4,000	α-mouse antibody α-rabbit antibody	Yes

Western ReProbe™

For multiple probing of Western blots

A single component system, specifically formulated to dissociate and remove antibodies from membrane bound proteins without destroying the antigenic binding affinity and does not use denaturants, SDS or boiling. Western ReProbe™ allows you the ability to reuse your Western blots. The stripped blots can then be probed with new probes.

Western ReProbe™ is not recommended for stripping color producing Western blots that use substrates such as TMB, chloronapthol and DAB. Supplied as a 5X solution; uses 15-20ml for each standard (7.5 x 8.5cm) Western blots.

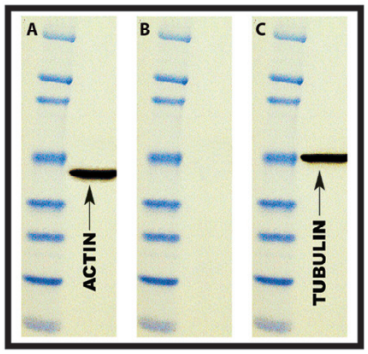


Figure 16: Mouse liver extract was transferred onto PVDF membrane and first probed for actin, then stripped with Western ReProbe™ and subsequently screened for tubulin antigens. Tubulin band was developed without loss of signal or background problems.

FEATURES

- Simply incubate at room temperature and wash
- No boiling, denaturants or SDS required

APPLICATIONS

- Reprobe for housekeeping proteins
- Compare phosphorylated and total protein on the same blot
- Re-analysis and correction of unsatisfactory Western blots
- Conservation of hard-to-obtain test samples and reagents

CITED REFERENCES

Ebrahem, Q. et al (2011) Invest. Ophthalmol. Vis. Sci. 52:6117
George, J. et al (2010) Neuro Oncol. 12:1088
Qi, J. et al (2009) J. Biol. Chem. 284: 19927 - 19936
George, J. et al (2009) Clin. Cancer Res. 15: 7186 - 7195
Di, X. et al (2008) Hum. Reprod. 23: 1873-83
Roy, S. and Tenniswood, M. (2007) J. Biol. Chem. 282: 4765
George, J. et al (2007) Clin Cancer Res. 13(12): 3507-17
Roy, S. and Tenniswood, M. (2007) J. Biol. Chem., 282: 4765 - 4771
Brown, K. et al (2006) Am. J. Physiol. Lung 290: 259
Joshi, M. et al (2006) Amer J. Respir. 10.1164.
Bu, S. et al (2006) Reproduction 131:1099.
Lam, A.M.I and Frick, D.N. (2006) J. Virol. 80: 404
Roy, S. et al (2005) Cell Death Differ. 12: 482
Li, Q. et al (2004) Reproduction 128:555
Bakke, L.J. et al (2004) Biol. Reprod. 71:605
Rubin, M. et al (2004) Cancer Res. 64: 3814
Small, G. et al (2004) Mol. Pharmacol. 66: 1478
Small, G. et al (2003) J. Pharmacol. Exp. Ther. 307: 861
Kobori, H. et al (2003) Hypertension 41: 592
Kobori, H. et al (2003) Hypertension 41: 42
Schraufstatter, I. et al (2003) J. Immunol. 171: 6714
Kuefer, R. et al (2002) Am. J. Path. 161: 841
Muthumani, K. et al (2002) JBC 277: 37820
Dash, A. et al (2002) Am. J. Path. 161: 1743
Orlawski, R. et al (2002) J. Biol. Chem. 277: 27864
Schraufstatter, I. et al (2002) J. Immunol. 169: 2102
Zhang, Y. et al (2001) Mol. Endocrinol. 15: 1891

Cat. No.	Description	Size
786-119	Western ReProbe™ [5X]	100ml
786-305	Western ReProbe™ [5X]	500ml
786-306	Western ReProbe™ [5X]	1L

Western ReProbe™ PLUS

Remove high affinity antibodies

Based on our popular Western ReProbe™, the modified formulation allows for the removal of stubborn, high affinity antibodies from membrane bound proteins without destroying the antigenic binding affinity. Not recommended for stripping color producing Western blots that use substrates such as TMB, chloronapthol and DAB. Requires no dilution and uses 15-20ml for each standard (7.5 x 8.5cm) Western blots.

FEATURES

- Ready-to-use, no dilution required
- Simply incubate at room temperature and wash
- No boiling, denaturants or SDS required

APPLICATIONS

- Removes high affinity antibodies
- Reprobe for housekeeping proteins
- Compare phosphorylated and total protein on the same blot
- Re-analysis and correction of unsatisfactory Western blots
- Conservation of hard-to-obtain test samples and reagents

Cat. No.	Description	Size
786-307	Western ReProbe™ PLUS	500ml
786-308	Western ReProbe™ PLUS	1L
786-309	Western ReProbe™ PLUS	1gal

FILM CLEANER

Swift™ Film Cleaner

Clean spotty or overexposed film

Cleans film that has been overexposed or have a high background/speckling without having to repeat experiments. Suitable for all exposed film developed for gel shift assays, Western, Southern and Northern blots.

FEATURES

- Save time by eliminating need to repeating experiment
- Provides even signal removal to preserve results
- Rapidly stopped once optimal signal achieved

Cat. No.	Description	Size
786-678	Swift™ Film Cleaner	Makes 2.4 L

Uniform High Background

Suggested Cause	Resolution/ Precaution
Concentration of antibody too high	• Reduce the concentration of primary and/ or secondary antibodies as high concentration can result in a high background.
Interference from incompatible blocking agent	• Investigate a different blocking agent, such as non animal protein blocking agents.
Antibodies cross-react with proteins present in blocking agents	• Investigate a different blocking agent, such as non animal protein blocking agents. • Avoid milk based blocking agents when probing with avidin/biotin systems. Milk contains biotin.
Non-specific sites insufficiently blocked	• Optimize the blocking buffer and conditions, including amount and type of blocking protein (agent) and length and temperature of blocking step. • Add Tween® 20 to the blocking agent, if detergent is not already present. Final concentration of 0.05%. • Incubate with antibodies in blocking agent containing 0.05% Tween® 20.
Washing steps insufficient	• Increase volume and length of wash steps. • Use wash buffers with Tween® 20, such as out femto-TBST™ or femto-TBST™ Wash Buffers.
Membrane exposed too long to film	• Reduce the exposure time.
Membrane issues	• Membranes not wetted correctly, check manufacturer's instructions. • Membrane inadvertently dried out during procedure. • Use orbital shaking or rocking with all incubation steps. • Handle membrane carefully, do not touch with exposed skin.
Bacterial or other contamination	• Prepare fresh buffers.

Blotchy or Speckled High Background

Suggested Cause	Resolution/ Precaution
Concentration of antibody too high	• Reduce the concentration of primary and/ or secondary antibodies as high concentration can result in a high background.
Interference from incompatible blocking agent	• Investigate a different blocking agent, such as non animal protein blocking agents.
HRP conjugate may have aggregated	• Filter through a 0.2µm filter. • Use new conjugate.
Antibodies cross-react with proteins present in blocking agents	• Investigate a different blocking agent, such as non animal protein blocking agents. • Avoid milk based blocking agents when probing with avidin/biotin systems. Milk contains biotin.
Non-specific sites insufficiently blocked	• Optimize the blocking buffer and conditions, including amount and type of blocking protein (agent) and length and temperature of blocking step. • Add Tween® 20 to the blocking agent, if detergent is not already present. Final concentration of 0.05%. • Incubate with antibodies in blocking agent containing 0.05% Tween® 20.
Washing steps insufficient	• Increase volume and length of wash steps. • Use wash buffers with Tween® 20, such as out femto-TBST™ or femto-TBST™ Wash Buffers.
Membrane exposed too long to film	• Reduce the exposure time.
Membrane issues	• Membranes not wetted correctly, check manufacturer's instructions. • Membrane inadvertently dried out during procedure. • Use orbital shaking or rocking with all incubation steps. • Handle membrane carefully, do not touch with exposed skin.
Bacterial or other contamination	• Prepare fresh buffers.
Dirty equipment	• Ensure all equipment is free of contaminants. • Ensure no residual gel pieces are present on the membrane.

Weak or No Signal

Suggested Cause	Resolution/ Precaution
Improper transfer of proteins to membrane	• Ensure correct protein transfer by staining the membrane with a suitable, reversible membrane stain. We recommend Swift™ Membrane Stain (Cat. No. 786-677). • For poor transfer of high molecular weight proteins, use our High Molecular Weight Transfer Buffer (Cat. No. 786-423). • Ensure the transfer sandwich and apparatus is assembled correctly and electrodes are correctly orientated. • Avoid over heating during transfer.
Poor binding of protein to membrane	• Add 20% methanol to transfer buffer for improved binding. • For low molecular weight proteins, reduce transfer time or use a membrane with a smaller pore size to prevent proteins passing through.
Concentration of antibody too high	• Reduce the concentration of primary and/ or secondary antibodies as high concentration can result in the signal generating too quickly and fading away before development.
Concentration of antibody too low	• Increase antibody concentration to overcome possible poor affinities.
Antigen levels too low	• Load more proteins on the inital gel.
Blocking agent binds antigen	• Optimize blocking agent type and concentration.
HRP activity inhibited by sodium azide	• Avoid using buffers that use sodium azide as a preservative.
Exposure time too short	• Extend the film exposure time.
Detection substrate inactive	• Ensure the substrate(s) shelf life has not expired. • Cross reaction between the 2/3 component systems may have occured. • Check for activity by preparing substrate and, in a dark room, add a small ampount of conjugate. If active a blue light should appear.
Excessive stripping	• If the membrane has been stripped, antigen sites may have been destroyed. • Use mild stripping conditions, we recommend Western ReProbe™ (Cat. No. 786-119). • Limited the number of times a membrane is reprobed.

Non-Specific Bands

Suggested Cause	Resolution/ Precaution
Concentration of antibody too high	• Reduce the concentration of primary and/ or secondary antibodies as high concentration can result in non-specific binding.
Presence of SDS	• Thoroughly wash blot after transfer • Do not use SDS in development steps

Diffuse Bands

Suggested Cause	Resolution/ Precaution
Concentration of antibody too high	• Reduce the concentration of primary and/ or secondary antibodies as high concentration can result in non-specific binding.
Protein concentration too high	• Reduce the amount of protein loaded

Ghost/ Hollow Bands or Brown/Yellow Bands on Membrane

Suggested Cause	Resolution/ Precaution
Concentration of antibody too high	• Reduce the concentration of primary and/ or secondary antibodies as high concentration can result in rapid consumption of the substrate.

Blank Areas

Suggested Cause	Resolution/ Precaution
Incomplete transfer of proteins	• Ensure correct protein transfer by staining the membrane with a suitable, reversible membrane stain. We recommend Swift™ Membrane Stain (Cat. No. 786-677). • Ensure the transfer sandwich and apparatus is assembled correctly and electrodes are correctly orientated. • Avoid over heating during transfer.

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

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

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

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

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

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

CELL/TISSUE LYSIS

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

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

PROTEIN EXTRACTION & LYSIS BUFFER (PE LB™) SYSTEMS

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

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

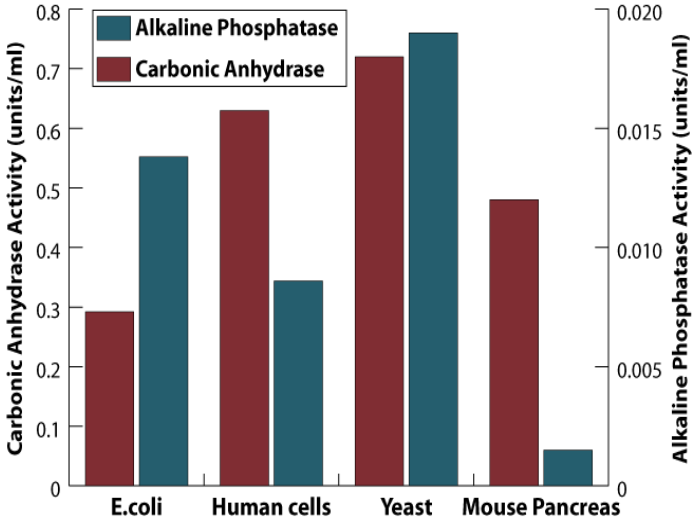


Figure 17: PE LB™ System maintains the biological activity of proteins. Extraction of carbonic anhydrase or alkaline phosphatase from E.coli, human cells, yeast and mouse pancreas with Bacterial, Mammalian Cell, Yeast and Tissue PE LB™ respectively. The resulting lysates were submitted to enzyme assays 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.

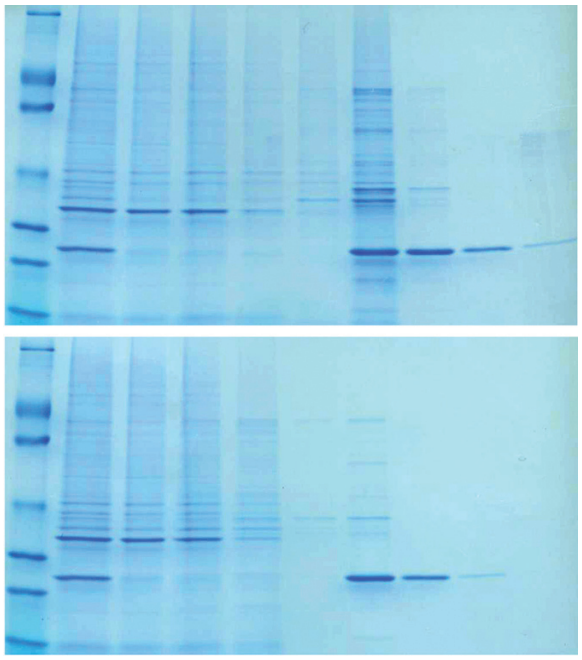


Figure 18: Bacteria expressing a His-tagged protein were lysed with Bacterial PE-LB™ and the recombinant protein was purified with HOOK™ 6X His Protein Purification kits (Top: Nickel resin; Bottom: Cobalt resin). Lane 1: PAGEmark™ protein ladder; 2: Cleared lysate; 3: Flow through; 4-6: Washes; 7-9: Elutions.

FEATURES

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

APPLICATIONS

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

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

Yeast PE LB™

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

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

FEATURES

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

APPLICATIONS

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

CITED REFERENCES

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

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

Mammalian Cell PE LB™

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

FEATURES

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

APPLICATIONS

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

CITED REFERENCES

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

Tissue PE LB™

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

FEATURES

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

APPLICATIONS

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

CITED REFERENCES

Kavanagh, K. et al (2012) J Gerontol A Biol Sci Med Sci. 10:1093
Kavanaugh, K. et al (2011) Am J Physiol Endocrinol Metab. 300:E894
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. No.	Description	Size
786-181	Tissue PE LB™	500ml

Insect PE LB™

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

FEATURES

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

APPLICATIONS

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

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

MISCELLANEOUS LYSIS PRODUCTS

Total Protein Extraction (TPE™)

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

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

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

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

FEATURES

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

APPLICATIONS

- Suitable for solubilization of total proteins for electrophoresis

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

RIPA Lysis & Extraction Buffer

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

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

CITED REFERENCES

Zhang, L. et al (2011) Am J Physiol Endocrinol Metab. 301: E599

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

IBS™ Buffer

Inclusion bodies solubilization buffer

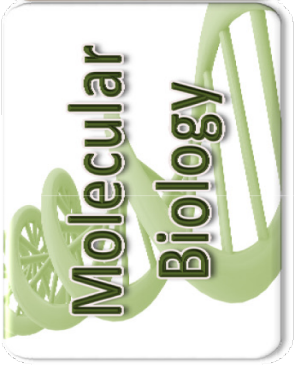
The expression of recombinant proteins is a popular and routinely used technique in protein studies. The expression of recombinant proteins often has one drawback and that is the recombinant proteins aggregate and form inclusion bodies, especially when expressed at high levels. The aggregated proteins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

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

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

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

G-Biosciences Product Line Overview



Estimation	7 Assays	CB-X Non Interfering SPN RED 660 dotMETRIC BCA CB	
Isolation	Extraction & Lysis	Sample Grinding Lysis Buffers	Mild Denaturing Strong Chaotropic Specialized
	Fractionation & Enrichment	12 Fractionation Kits Dialysis (Micro) Concentration	
	Sample Preparation	Contamination Removal	Desalting Detergent Removal General Cocktails Species Specific Individual Inhibitors
	Reagents	Protease Inhibitors Detergents Chaotropes	
Detection	Electrophoresis	1D & 2D Reagents Gel Stains	2D Specific Kits Buffers & Reagents Coomassie Silver Reversible
	Western Blotting	1 Hour System Blocking Agents	Non-Animal Animal Non-Protein
		Secondary Antibodies Chemiluminescence Detection	
	Mass Spectrometry	Trypsin, Mass Spec Grade InGel Kits	
	Assays (ELISA)	Coated Plates Blocking Agents	Non-Animal Animal Non-Protein
		Secondary Antibodies Detection Reagents	
Purification	Affinity Resins	6X His Tag	Nickel resin Cobalt resin Copper resin Zinc Resin Glutathione Resin Streptavidin Resin Calmodulin Resin
		GST Tag Biotin Tag CBP Tag	
	Activated Resins	Sulfhydryl reactive Amine reactive Carboxyl reactive	
	Antibody Purification	Drug/ Steroid reactive Protein A or G Pearl Resin	
Modification	Labeling	Biotin Fluorescent Dye Enzyme (HRP/AP)	
	Crosslinkers Reducing Agents Alkylating Agents Protein Cleavage Iodination Amino Acid Side Chain Modifiers		
Antibody	Production	Carrier Proteins	BSA KLH HyperCarrier
	Purification	Peptide Coupling Protein A or G Resin Activated Resins Pearl Resin Thiophilic Resin	
	Fragmentation	Ficin Pepsin Papain	
SAM Methyltransferase	Continuous, Enzymatic Assays Lactate Dehydrogenase (LDH)		
Cell Toxicity & Proliferation	SRB WST-1		
Apoptosis	Caspase	Assays Substrates Inhibitors	
	Inducers		
Protease	Assays Inhibitors		
Phosphatase Peroxide			
B-Galactosidase	CPRG Fluorescent (MUG)		
Genomic DNA	Isolation	Tissue Blood Plant Yeast Bacteria Fungi Mouse Tail	
Plasmid DNA	Isolation Colony Screening Transformation		
Electrophoresis	Apparatus Loading Dyes DNA Ladders Gel Extraction		
PCR	Taq dNTPs		
RNA	Extraction RNase Decontamination		
Yeast	Transformation Plasmid Isolation		

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