Novex® Pre-Cast Gel Electrophoresis Guide

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Novex® Pre-Cast Gel Electrophoresis Guide

General information and protocols for using Novex® pre-cast gels

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General Information

Purpose of the Guide

The Novex® Pre-Cast Gel Electrophoresis Guide contains information about the Novex® Pre-Cast gels and is intended to supplement the Gel Instruction Cards (IM-6000 to IM-6008) supplied with the pre-cast gels. Complete protocols for sample and buffer preparation, electrophoresis conditions, staining, and blotting are provided in this guide.

To request the instruction cards or for additional information, call Technical Service (see page 66) or download the manuals from our Web site at www.invitrogen.com.

Storage and Shelf life

Store Novex® Pre-Cast Gels at +4°C. The gels have a shelf life of 4-8 weeks depending upon the gel type when stored at +4°C.

Do not freeze Novex® Pre-Cast Gels.

Use gels immediately from the refrigerator. Extended exposure of the gels to room temperature seriously impairs the performance of the gel.

Packaging

The Novex® Pre-Cast Gels are supplied as 10 gels per box. Gels are individually packaged in clear pouches with 4-10 ml of Packaging Buffer.

Handling the Gels

The Packaging Buffer contains 0.02% sodium azide and residual acylamide monomer. Wear gloves at all times when handling gels.

Warning: This product contains a chemical (acrylamide) known to the state of California to cause cancer. Refer to the MSDS (see page 66).

Introduction

Overview of Electrophoresis

Introduction

Electrophoresis is defined as the transport of charged molecules through a solvent by an electrical field. Electrophoresis is a simple, rapid, and sensitive analytical tool for separating proteins and nucleic acids.

Any charged ion or molecule will migrate when placed in an electrical field. Most biological molecules carry a net charge at any pH other than their isoelectric point and will migrate at a rate proportional to their charge density.

The mobility of a biological molecule through an electric field will depend on the following factors:

- Field strength
- Net charge on the molecule
- Size and shape of the molecule
- Ionic strength and properties of the medium through which the molecules migrate

Support Matrix

Two types of support matrices are used in electrophoresis. These include polyacrylamide and agarose. The support matrix acts a porous media and behaves like a molecular sieve. The molecular sieving function of the matrix depends on the gel pore size of the matrix. Agarose has a large pore size and is ideal for separating macromolecules such as nucleic acids and protein complexes. Polyacrylamide has a smaller pore size and is ideal for separating most proteins and smaller nucleic acids.

Polyacrylamide Gel Electrophoresis (PAGE)

Polyacrylamide gels are generated by the polymerization of acrylamide monomers into long chains and the crosslinking of these long chains by bifunctional compounds such as N,N-methylene-bisacrylamide (bis) reacting with the free functional groups at chain termini.

The concentration of acrylamide and bisacrylamide (%T and %C) determines the pore size of the gel.

%T=concentration of total monomer

%C=proportion of cross linker (as a percentage of total monomer)

The higher the acrylamide concentration, the smaller the pore size, resulting in resolution of low molecular weight molecules and vice-versa.

Continued on next page

Overview of Electrophoresis, Continued

Buffer Systems

Electrophoresis is performed using continuous or discontinuous buffer systems. A continuous buffer system utilizes only one buffer in the gel and the running buffer.

A discontinuous buffer system (Ornstein 1964) utilizes different gel buffer and running buffer. This buffer system also uses at least two gel layers of different pore sizes, the stacking and separating gel. Electrophoresis using a discontinuous buffer system results in concentration of the sample and higher resolution.

Electrophoresis Conditions

The separation of molecules will depend on the electrophoresis conditions. Electrophoresis can be performed under the following conditions:

Denaturing Conditions

Electrophoresis is performed under denaturing conditions using an anionic detergent such as sodium dodecylsulfate (SDS). SDS denatures and unfolds the proteins by wrapping around the hydrophobic portions of the protein. SDS binds at a ratio of ~1.4 g SDS per gram of protein. The resultant SDS-protein complexes are highly negatively charged and migrate through the gel based on their size rather than charge.

Non-Denaturing (Native) Conditions

Electrophoresis is performed under non-denaturing (native) conditions using buffer systems that maintain the native protein confirmation, subunit interaction, and biological activity. During native electrophoresis, proteins are separated based on their charge to mass ratios.

Reducing Conditions

Electrophoresis is performed under reducing conditions using reducing agents such as dithiothreitol (DTT) or β-mercaptoethanol (β-ME). The reducing agents completely unfold the denatured proteins into their subunits by cleaving the disulfide bonds between cysteine residues.

Continued on next page

Power Considerations for Electrophoresis

Electrophoresis is based on the following two equations:
Voltage = Current x Resistance (V=IR)
Wattage = Current x Voltage (W=IV)

Resistance

Resistance of the assembled electrophoresis cell is dependent on the conductivity of the gel buffer, the thickness of the gel, and the number of gels being run. Although the resistance is determined by the gel system, the resistance varies over the course of the run. In the Tris-Glycine buffer system, the fast moving, highly conductive chloride ions in the gel are gradually replaced by the slower moving, less conductive glycine ions from the running buffer as the gel runs. As a result, the resistance of the gel increases as the chloride/glycine front moves down the gel, and the current decreases.

Constant Voltage

The velocity in which an ion moves in an electric field will vary in proportion to the field strength (Volts per unit distance). The higher the voltage the faster an ion will move. For most electrophoresis applications, we recommend constant voltage setting. Using constant voltage provides the following advantages:

- Current and watts decrease throughout the run, providing a safety margin.
- The same voltage setting can be used regardless of the number or thickness of gels being electrophoresed.

Constant Current

Current is a function of the number of ions passing a given cross-section of the circuit at a given time. For a given gel/buffer system, at a given temperature, current will vary in proportion to the field strength (voltage) and/or cross-sectional area (number and/or thickness of the gels). Ions in solution and at a given voltage will move faster as the temperature increases, increasing current. Discontinuous buffer systems and, to a lesser extent continuous buffer systems, increase resistance during the run. If you use constant current setting on the power supply, the voltage will increase as resistance increases to satisfy Ohm's law (V=IR). If no voltage limit is set and a local fault condition occurs, such as a poor connection, very high local resistance may cause the voltage to increase to a maximum of the power supply. This will lead to local overheating and damage the electrophoresis cell or create unsafe conditions.

When running under constant current conditions, set a voltage limit on the power supply at or slightly above the maximum expected voltage.

Constant Power

Watts, or the rate of heat generated by the system, is a function of voltage and current (W=IV). For a given gel system if voltage is doubled, watts will also double (as V=IR, and R is a "constant" determined by the gel system). If power is constant, voltage will increase and current will decrease during a run, but the total amount of heat generated by the system will remain constant throughout the run. However, locally high resistance can cause a high proportion of total heat to be generated over a small distance. This can damage the electrophoresis cell and/or gel(s). If operating at a constant power, set the voltage limit to slightly above the maximum expected for the run.

Novex® Pre-Cast Gels

Introduction

A large variety of pre-cast gels are available from Invitrogen. These include gels for analysis of proteins (Tris-Glycine, Tricine, Zymogram, IEF, and ZOOM® Gels) and nucleic acids (TBE, TBE-Urea, and DNA Retardation).

General information on Novex® Pre-Cast Gels is provided in this section. Specifications and gel formulations are listed on pages 6-7. Information on each gel type is provided on pages 10-28. For ordering information on Novex® Pre-Cast Gels, visit our Web site at www.invitrogen.com or contact Technical Service (see page 66).

Novex® Pre-Cast Gels are capable of resolving proteins in the range of 2-500 kDa and nucleic acids in the range of 10-3000 bp.

Choosing a Gel for Your Application

To obtain the best results for your application, it is important to choose the correct gel percentage, buffer system, gel format, and thickness .

A variety of factors affect the choice of a gel. These include:

Application

Based on the type of your application, you can choose from gels for protein separation (Tris-Glycine, Tricine, IEF, ZOOM®, and Zymogram Gels) or gels for nucleic acid separation (TBE, TBE-Urea, and DNA Retardation Gels). See next page for more details.

Size of the molecule being separated

Large molecules resolve well on a low percentage gels while small molecules are best resolved on high percentage gels. The size of the molecule usually dictates the acrylamide percentage. If you do not know the molecular weight of the molecule or are separating a wide molecular weight range of molecules, choose gradient gels.

Amount of available material

The higher the number of wells and the thinner the gel, the lower the sample loading volume and vice versa (see page 6 for the recommended loading volumes for the various well formats). Based on the amount of your starting material available, you can choose from a variety of comb types. **Note:** Proteins will transfer more easily out of a 1.0 mm gel than a 1.5 mm gel.

Refer to the Gel Migration Chart on our Web site at www.invitrogen.com or in our catalog to choose the right gel for your application. Choose a gel such that the molecules migrate about 70% of the length of gel for best resolution (gray shaded area on the Gel Migration Chart).

Compatibility

The size of a Novex® Pre-Cast Gel is 10 x 10 cm (gel size is 8 x 8 cm). We recommend using the XCell *SureLock*™ Mini-Cell (see page 57 for ordering information) for the electrophoresis of Novex® Pre-Cast Gels to obtain optimal and consistent performance. Novex® Pre-Cast Gels are compatible with most other mini-cells designed for electrophoresis of 10 cm (h) x 10 cm (w) gel cassettes.

Continued on next page

Staining Novex® Pre-Cast Gels

The Novex® Pre-Cast Gels are compatible with most silver staining protocols. We recommend using the SilverQuest™ Silver Staining Kit or the SilverXpress® Silver Staining Kit (see pages 33-37) for silver staining of Novex® Gels.

The Novex® Pre-Cast Gels are compatible with any of the standard Coomassie® staining procedures. The protocols that are accelerated by heat are preferable as heat serves as a "fix" for proteins, especially smaller peptides. The SimplyBlue™ SafeStain and Novex® Colloidal Coomassie® Blue Staining Kit (see pages 38-41) are recommended for staining Novex® Gels.

Applications

Separating proteins over a wide range of molecular weights

The Novex® Tris-Glycine Gels are used for separating proteins over a wide molecular weight range under denaturing or non-denaturing conditions.

Resolving low molecular weight proteins and peptides

The Novex® Tricine Gels provide high resolution of low molecular weight proteins and peptides.

Performing Isoelectric focusing (IEF)

Use Novex® IEF Gels for native (vertical) IEF of proteins.

Detecting Proteases

The Novex® Zymogram Gels are used for detecting and characterizing proteases that utilize casein or gelatin as the substrate.

Performing 2D Separation of Proteins

The ZOOM® Gels are specifically designed for second dimension electrophoresis of 7.0 cm IPG strips.

Performing Nucleic Acid Analysis

The Novex® TBE Gels are used to analyze DNA fragments including restriction digest, PCR products, Southern analysis, and primer analysis. The Novex® TBE-Urea Gels are used for denaturing nucleic acid analysis and are suited for RNase Protection Assays, *in-vitro* transcription studies, RNA stability studies, and oligonucleotide purification.

Performing Gel Shift Assays

The Novex® 6% DNA Retardation Gels are used to perform gel shift assays.

Novex® Pre-Cast Gel Specifications

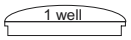
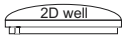
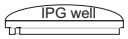
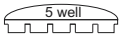
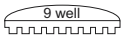
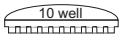
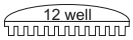
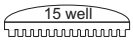
Specifications

Gel Matrix:	Acrylamide/Bisacrylamide
Gel Thickness:	1.0 mm or 1.5 mm
Gel Size:	8 cm x 8 cm
Cassette Size:	10 cm x 10 cm
Cassette Material:	Styrene Copolymer (recycle code 7)
Sample Well Configuration	1, 5, 9, 10, 12, 15-well, 2D -well, and IPG well

Recommended Loading Volumes

The recommended loading volumes and protein load per band by the detection method are provided in the table below.

Note: The 9-well gels are compatible with any eight-channel pipettors used for loading samples from 96-well plates. An additional lane is included for loading protein molecular weight standard.

Well Types	Maximum Load Volume	Maximum Protein Load Per Band by Detection Method		
		Coomassie® Staining	**Ethidium Bromide**	**Silver Staining**
 1.0 mm	700 µl	12 µg/band	2.4 µg/band	Scale your sample load for the sensitivity of your silver staining kit. For use with the SilverQuest™ or SilverXpress ® Silver Staining Kits, we recommend a protein load of 1 ng/band.
 1.0 mm 1.5 mm	400 µl 600 µl	12 µg/band	2.0 µg/band	
 1.0 mm	7 cm IPG Strip	N/A	N/A	
 1.0 mm	60 µl	2 µg	400 ng/band	
 1.0 mm	28 µl	0.5 µg/band	100 ng/band	
 1.0 mm 1.5 mm	25 µl 37 µl	0.5 µg/band	100 ng/band	
 1.0 mm	20 µl	0.5 µg/band	100 ng/band	
 1.0 mm 1.5 mm	15 µl 25 µl	0.5 µg/band	100 ng/band	

Novex® Pre-Cast Gel Formulations

Gel Formulations

All Novex® Pre-Cast gels are made with high purity reagents. The gels for DNA analysis are DNase and RNase-free. The composition of the different gels is listed below:

Gel Type	Formulation	Stacking Gel	Separating Gel	% Bis-Acrylamide	pH
Tris-Glycine Gels (except 4%)	Tris-base, HCl, Acrylamide, Bis-acrylamide, TEMED, APS, Ultrapure water	4%	6%, 8%, 10%, 12%, 14%, 16%, 18%, 4-12%, 8-16%, 4-20%, 10-20%	2.6%	8.6
4% Tris-Glycine Gels	Same as Tris Glycine	3.5%	4%	1.3%	8.6
Tricine Gels	Tris-base, HCl, Acrylamide, Bis-acrylamide, TEMED, APS, Ultrapure water	4%	10%, 16%, 10-20%	2.6%	8.3
Zymogram Gels	Tris Glycine Gels with a substrate, casein or gelatin	4% No substrate	10%, 12%, 4-16%	2.6%	8.6
IEF Gels	Acrylamide, Bis-acrylamide, TEMED, APS, Ultrapure water, 2% ampholytes	None	pH 3-7 pH 3-10	2.6%	5.0 6.0
TBE Gels	Tris-base, Boric acid, EDTA, Acrylamide, Bis-acrylamide, TEMED, APS, Ultrapure water	4%	6%, 8%, 10%, 20%, 4-12%, 4-20%	2.6%	8.3
TBE-Urea Gels	Tris-base, Boric acid, EDTA, Acrylamide, Bis-acrylamide, TEMED, APS, Ultrapure water, 7M Urea	4%	6%, 10%, 15%	3.8-5%	8.7
DNA Retardation Gels	6% polyacrylamide gels prepared with half strength TBE gel buffer	None	6%	2.6%	8.3

Important

Novex® Pre-Cast gels do not contain SDS. These gels can be used for non-denaturing (native) and denaturing gel electrophoresis.

For optimal and total separation ranges for each specific gel percentage, consult the Gel Migration Chart on our Web site at www.invitrogen.com or the catalog.

Methods

General Guidelines for Preparing Samples and Buffers

Introduction

General guidelines for preparing samples and buffers for Novex® Pre-Cast gels are discussed below.

Detailed instructions for preparing the sample buffer and running buffer for each gel type are provided on pages 10-28.

Recommended Buffers

The recommended running buffer and sample buffer for each Novex® Pre-Cast Gel is listed in the table below. You will prepare your sample in the appropriate sample buffer so the final concentration of the sample buffer will be 1X. You will need to dilute the running buffer to 1X final concentration before use.

See page 57 for ordering information on pre-mixed buffers and page 59 for recipes if you are making your own buffers.

Gel Type	Running Buffer	Sample Buffer
Tris-Glycine Gel (SDS-PAGE)	Tris-Glycine SDS Running Buffer (10X)	Tris-Glycine SDS Sample Buffer (2X)
Tris-Glycine (Native-PAGE)	Tris-Glycine Native Running Buffer (10X)	Tris-Glycine Native Sample Buffer (2X)
Tricine Gels	Tricine SDS Running Buffer (10X)	Tricine SDS Sample Buffer (2X)
Zymogram Gels	Tris-Glycine SDS Running Buffer (10X)	Tris-Glycine SDS Sample Buffer (2X)
IEF Gels	IEF Cathode Buffer (10X) IEF Anode Buffer (50X)	IEF Sample Buffer (2X)
TBE Gels	TBE Running Buffer (5X)	Hi-Density TBE Sample Buffer (5X)
TBE-Urea Gels	TBE Running Buffer (5X)	TBE-Urea Sample Buffer (2X) Prep TBE-Urea Sample Buffer (2X) for preparative gels
DNA Retardation Gels	TBE Running Buffer (5X)	Hi-Density TBE Sample Buffer (5X)

Reducing Agent

The NuPAGE® Reducing Agent contains 500 mM dithiothreitol (DTT) at a 10X concentration in a ready-to-use, stabilized liquid form (see page 57 for ordering information) and is used to prepare samples for reducing gel electrophoresis.

β-mercaptoethanol can be used with the Novex® gels at a final concentration of 2.5%. Choice of the reducing agent is a matter of preference and either DTT or β-mercaptoethanol can be used. **We recommend adding the reducing agent to the sample within an hour of loading the gel.**

Avoid storing reduced samples for long periods even if they are frozen. This will result in the reoxidation of samples during storage and produce inconsistent results.

Continued on next page

General Guidelines for Preparing Samples and Buffers,
Continued

Running Reduced and Non-Reduced Samples

For optimal results, we do not recommend running reduced and non-reduced samples on the same gel.

If you do choose to run reduced and non-reduced samples on the same gel, do not run reduced and non-reduced samples in adjacent lanes. The reducing agent may have a carry-over effect on the non-reduced samples if they are in close proximity.

Heating Samples

Heating the sample at 100°C in SDS containing buffer results in proteolysis (Kubo, 1995). We recommend heating samples for denaturing electrophoresis (reduced or non-reduced) at 85°C for 2-5 minutes for optimal results.

Do not heat the samples for non-denaturing (native) electrophoresis or Zymogram Gels.

Tris-Glycine Gels

Introduction

The Tris-Glycine gels are based on the Laemmli System (Laemmli, 1970) with minor modifications for maximum performance in the pre-cast format. The separating and stacking gels of Novex® Tris-Glycine gels have a pH of 8.65 unlike traditional Laemmli gels that have a stacking gel pH of 6.8 and separating gel pH of 8.8.

The separating range of Tris-Glycine gels is 6-200 kDa.

Tris-Glycine Discontinuous Buffer System

The Tris-Glycine discontinuous buffer systems involves three ions:

- Chloride ($^-$) is supplied by the gel buffer and serves as a leading ion due to its high affinity to the anode as compared to other anions in the system. The gel buffer ions are $Tris^+$ and Cl^- (pH 8.65).
- Glycine ($^-$) is the primary anion supplied by the running buffer and serves as a trailing ion. Glycine is partially negatively charged and trails behind the highly charged chloride ions in the charged environment. The running buffer ions are $Tris^+$, Gly^-, and $dodecylsulfate^-$ (pH 8.3).
- Tris Base ($^+$) is the common ion present in the gel buffer and running buffer. During electrophoresis, the gel and buffer ions in the Tris-Glycine system form an operating pH of 9.5 in the separation region of the gel.

Materials Supplied by the User

You will need the following items. Ordering information is on page 57 and recipes are provided on page 59 if you are preparing your own buffers.

- Protein sample
- Deionized water
- Protein molecular weight markers

For denaturing electrophoresis

- Tris-Glycine SDS Sample Buffer
- NuPAGE® Reducing Agent
- Tris-Glycine SDS Running Buffer

For non-denaturing electrophoresis

- Tris-Glycine Native Sample Buffer
- Tris-Glycine Native Running Buffer

Continued on next page

Tris-Glycine Gels, Continued

Preparing Sample

The Novex® Tris-Glycine SDS or Native Sample Buffer (2X) and NuPAGE® Reducing Agent (10X) are available from Invitrogen (see page 57 for ordering information).

1. Prepare denatured or non-denatured (native) samples for Tris-Glycine gels as described below:

   Note: For reduced samples, add the reducing agent to a final concentration of 1X immediately prior to electrophoresis to obtain the best results.

Reagent	Denatured Sample	Native Sample
Sample	x µl	x µl
Tris-Glycine SDS Sample Buffer (2X)	5 µl	--
Tris-Glycine Native Sample Buffer (2X)		5 µl
Deionized Water	to 5 µl	to 5 µl
Total Volume	10 µl	10 µl

2. Heat the sample at 85°C for 2 minutes. Load the sample immediately on the gel. **Do not heat samples for native electrophoresis.**

See page 60 for a recipe of the sample buffer, if you are preparing the sample buffer.

Preparing Running Buffer

Novex® Tris-Glycine SDS or Native Running Buffer (10X) is available from Invitrogen (see page 57 for ordering information).

1. Prepare 1000 ml of 1X Tris-Glycine SDS or Native Running Buffer using Novex® Tris-Glycine SDS or Native Running Buffer (10X) as follows:

Novex® Tris-Glycine SDS or Native Running Buffer (10X)	100 ml
Deionized Water	900 ml
Total Volume	1000 ml

2. Mix thoroughly. Use this buffer to fill the Upper and Lower Buffer Chambers of the XCell *SureLock*™ Mini-Cell for electrophoresis.

See page 59 for a recipe of the Novex® Tris-Glycine SDS and Native Running Buffers, if you are preparing the running buffers.

Electrophoresis Conditions

Instructions for running the Tris-Glycine gel using XCell *SureLock*™ Mini-Cell and the electrophoresis conditions are provided on page 30.

Tricine Gels

Introduction

Novex® Tricine Gels are ideal for peptides and low molecular weight proteins (less than 10 kDa). The Tricine Gels are based on the Tricine system developed by (Schaegger and vonJagow, 1987). In this buffer system, tricine substitutes glycine in the running buffer resulting in more efficient stacking and destacking of low molecular weight proteins and higher resolution of smaller peptides (see below). The Novex® Tricine Gels do not contain tricine in the gel, the tricine is supplied by the running buffer.

Tricine gels must be used with denatured or reduced proteins only. The separating range of Tricine gels is 2.5-200 kDa.

Advantages of Tricine Gels

The Tricine Gels have the following advantages over the Tris-Glycine Gels for resolving proteins in the molecular weight range of 2-20 kDa:

- Allows resolution of proteins with molecular weights as low as 2 kDa
- Ideal for direct sequencing of proteins after transferring to PVDF as tricine does not interfere with sequencing
- Minimizes protein modification as the Tricine buffer system has a lower pH

The Tricine System

The Tricine system is a modification of the Tris-Glycine discontinuous buffer system (see page 10) specifically designed for the resolution of low molecular weight proteins.

In the Tris-Glycine system, the proteins are stacked in the stacking gel between a highly mobile leading chloride ion (in the gel buffer) and the slower trailing glycine ion (in the running buffer). These stacked protein bands undergo sieving once they reach the separating gel.

However, the resolution of smaller proteins (<10 kDa) is hindered by the continuous accumulation of free dodecylsulfate (DS) ions (from the SDS sample and running buffers) in the stacking gel. This zone of stacked DS micelles causes mixing of the DS ions with the smaller proteins resulting in fuzzy bands and decreased resolution. The mixing also interferes with the fixing and staining of smaller proteins.

To solve this problem, the Tricine system uses a low pH of the gel buffer and replaces the trailing glycine ion with a fast moving tricine ion in the running buffer. The smaller proteins that migrate with the stacked DS micelles in the Tris-Glycine system are now well separated from DS ions in the Tricine system resulting in sharper bands and higher resolution.

Continued on next page

Tricine Gels, Continued

Materials Supplied by the User

You will need the following items. Ordering information is on page 57 and recipes are provided on page 61, if you are preparing your own buffers.

- Protein sample
- Deionized water
- Protein molecular weight markers
- Tricine SDS Sample Buffer
- NuPAGE® Reducing Agent for reduced samples
- Tricine SDS Running Buffer

Preparing Samples

The Novex® Tricine SDS Sample Buffer (2X) and NuPAGE® Reducing Agent (10X) are available from Invitrogen (see page 57 for ordering information).

1. Prepare reduced or non-reduced samples for Tricine gels as described below:

   Note: For reduced sample, add the reducing agent immediately prior to electrophoresis to obtain the best results.

Reagent	Reduced Sample	Non-reduced Sample
Sample	x µl	x µl
Novex® Tricine SDS Sample Buffer (2X)	5 µl	5 µl
NuPAGE® Reducing Agent (10X)	1 µl	--
Deionized Water	to 4 µl	to 5 µl
Total Volume	10 µl	10 µl

2. Heat samples at 85°C for 2 minutes. Load the sample immediately on the gel.

See page 61 for a recipe of the sample buffer, if you are preparing the sample buffer.

Preparing Running Buffer

Novex® Tricine SDS Running Buffer (10X) is available from Invitrogen (see page 57 for ordering information).

1. Prepare 1000 ml of 1X Tricine SDS Running Buffer using Novex® Tricine SDS Running Buffer (10X) as follows:

Novex® Tricine SDS Running Buffer (10X)	100 ml
Deionized Water	900 ml
Total Volume	1000 ml

2. Mix thoroughly. Use this buffer to fill the Upper and Lower Buffer Chambers of the XCell *SureLock*™ Mini-Cell for electrophoresis.

See page 61 for a recipe of the Novex® Tricine SDS Running Buffer, if you are preparing the running buffer.

Continued on next page

Note

If you accidentally run the Tricine Gel with a

- Tris-Glycine SDS Sample Buffer

  The bands will be poorly resolved.

- Tris-Glycine SDS Running Buffer

  The run will take longer time to complete and will result in poor resolution of smaller proteins.

Electrophoresis Conditions

Instructions for running the Tricine gel using the XCell *SureLock*™ Mini-Cell and the electrophoresis conditions are provided on page 30.

Zymogram Gels

Introduction

Novex® Zymogram Gels are used for detecting and characterizing metalloproteinases, collagenases, and various other proteases that can utilize casein or gelatin as a substrate. Proteases are easily visualized as clear bands against a dark blue background where the protease has digested the substrate.

The type and quantity of metalloproteinases expressed by tumor cells allow determination of the metastatic potential of the tumor.

Types of Zymogram Gels

The different types of Zymogram Gels available from Invitrogen are listed below:

- The Zymogram Gelatin Gel is a 10% Tris-Glycine gel with 0.1% gelatin incorporated as a substrate
- The Zymogram Casein Gel is a 12% Tris-Glycine gel with β-casein incorporated as a substrate
- The Zymogram Blue Casein Gel is a 4-16% Tris-Glycine gel with blue-stained β-casein incorporated as a substrate

Sensitivity Level

The sensitivity of the different Zymogram Gels is shown below:

10% Zymogram Gelatin Gel:	10^{-6} units of collagenase
12% Zymogram Casein Gel:	7×10^{-4} units of trypsin
4-16% Zymogram Blue Casein Gel:	1.5×10^{-3} units of trypsin

Zymogram Technique

The enzyme sample is denatured in SDS buffer (do not use reducing conditions) and electrophoresed on a Zymogram Gel **without heating** using the Tris-Glycine SDS Running Buffer. After the run is complete, the enzyme is renatured by incubating the gel in Zymogram Renaturing Buffer containing a non-ionic detergent. The gels are then equilibrated in Zymogram Developing Buffer (to add the divalent metal cation required for enzymatic activity) followed by staining and destaining the gel. The protease bands appear as clear bands against a dark background.

Materials Supplied by the User

You will need the following items. Ordering information is on page 57 and recipes are provided on page 62, if you are preparing your own buffers.

- Protein sample
- Deionized water
- Protein molecular weight markers
- Tris-Glycine SDS Sample Buffer
- Tris-Glycine SDS Running Buffer
- Zymogram Renaturing Buffer
- Zymogram Developing Buffer

Continued on next page

Zymogram Gels, Continued

Important

- Do not use reducing conditions to prepare samples for Zymogram Gels. Some of the proteases are multiunit complexes and require the subunit assembly for activity.
- If you are using protein molecular weight, load more of the unstained marker than on a Tris-Glycine gel as the marker needs to stain intensely to be visualized against the dark background.
- For protein molecular weight markers containing reducing agent, separate the marker from the protease sample lanes by an empty lane to prevent diffusion of the reducing agent which will interfere with the protease assay.

Preparing Samples

The Novex® Tris-Glycine SDS Sample Buffer (2X) is available from Invitrogen (see page 57 for ordering information).

1. Prepare samples for Zymogram gels as described below:

Reagent	Amount
Sample	x µl
Novex® Tris-Glycine SDS Sample Buffer (2X)	5 µl
Deionized Water	to 5 µl
Total Volume	10 µl

2. Load the sample immediately on the gel. **Do not heat samples for Zymogram Gels.**

See page 60 for a recipe of the sample buffer, if you are preparing the sample buffer.

Preparing Running Buffer

Novex® Tris-Glycine SDS Running Buffer (10X) is available from Invitrogen (see page 57 for ordering information).

1. Prepare 1000 ml of 1X Tris-Glycine SDS Running Buffer using Novex® Tris-Glycine SDS Running Buffer (10X) as follows:

Novex® Tris-Glycine SDS Running Buffer (10X)	100 ml
Deionized Water	900 ml
Total Volume	1000 ml

2. Mix thoroughly. Use this buffer to fill the Upper and Lower Buffer Chamber of the XCell *SureLock*™ Mini-Cell for electrophoresis.

See page 59 for a recipe of the Novex® Tris-Glycine SDS Running Buffer, if you are preparing the running buffer.

Electrophoresis Conditions

Instructions for running the Zymogram gel using XCell *SureLock*™ Mini-Cell and the electrophoresis conditions are provided on page 30.

Continued on next page

Zymogram Gels, Continued

Developing Zymogram Gels

Develop the Zymogram gel after electrophoresis to detect protease activity as follows:

1. Dilute the Novex® Zymogram Renaturing Buffer (10X) and Novex® Zymogram Developing Buffer (10X), 1:9 with deionized water. You will need 100 ml of each buffer per one or two mini-gels.
2. After electrophoresis, remove the gel and incubate the gel in the 1X Zymogram Renaturing Buffer from Step 1 for 30 minutes at room temperature with gentle agitation.
3. Decant the Zymogram Renaturing Buffer and add 1X Zymogram Developing Buffer to the gel.
4. Equilibrate the gel for 30 minutes at room temperature with gentle agitation.
5. Decant the buffer and add fresh 1X Zymogram Developing Buffer to the gel.
6. Incubate the gel at 37°C for at least 4 hours or overnight for maximum sensitivity. Incubation time can be reduced to 1 hour for concentrated samples. The optimal result is determined empirically by varying the sample load or incubation time.

Staining Zymogram Gels

Zymogram (Blue Casein) 4-16% gels do not require staining.

For non-pre-stained Zymogram gels, stain the gels with Colloidal Blue Staining Kit or the SimplyBlue™ Safestain as described on pages 38-41.

Areas of protease activity will appear as clear bands against a dark background.

IEF Gels

Introduction

IEF Gels are used to determine the isoelectric point (pI) of a protein and to detect minor changes in the protein due to posttranslational modifications such as phosphorylation and glycosylation. For details on isoelectric focusing, see below.

Novex® IEF Gels contain 5% polyacrylamide and are used for native applications. The pH 3-10 gels have a pI performance range of 3.5-8.5 and the pH 3-7 gels have a pI performance range of 3.0-7.0.

Proteins separated on IEF Gels are suitable for use in two-dimensional (2D) electrophoresis using a 2D-well format Novex® Tris-Glycine or NuPAGE® Gel.

Isoelectric Focusing (IEF)

Isoelectric focusing (IEF) is an electrophoretic technique for the separation of proteins based on their pI. The pI is the pH at which a protein has no net charge and will not migrate in an electric field.

In IEF, the proteins are applied to polyacrylamide gels (IEF Gels) or immobilized pH gradient (IPG) strips containing a fixed pH gradient. As the protein sample containing a mixture of different proteins migrates through the pH gradient, individual proteins are immobilized in the pH gradient as they approach their pI.

2D Electrophoresis

Two-dimensional (2D) gel electrophoresis is a powerful and sensitive technique for separating and analyzing protein mixtures from biological samples. 2D gel electrophoresis is performed in two consecutive steps:

1. First dimension separation of proteins using isoelectric focusing.

   Proteins are separated based on their isoelectric point or pI using IEF gels or IPG strips.

2. Second dimension separation of proteins using SDS-PAGE.

   Proteins are separated based on their molecular weight using denaturing polyacrylamide gel electrophoresis.

The second dimension gel is stained using an appropriate staining procedure to visualize the separated proteins as spots on the gel or the proteins are blotted onto membranes. Protein spots can be excised from the gel or membranes and subjected to further analyses such as mass spectrometry or chemical microsequencing to facilitate protein identification.

Power Considerations for IEF

To obtain the best results, IEF is typically performed by increasing the voltage gradually and maintaining the final focusing voltage for 30 minutes.

During IEF, proteins migrate in an electric field until a stable pH gradient is formed and proteins reach their pI. At that point, a high finishing voltage is applied to focus the proteins into narrow zones. High voltage cannot be applied during the initial stages of IEF due to excessive heat generated by the movement of carrier ampholytes.

Alternatively, IEF can be performed at constant wattage so the voltage will increase as the current decreases.

Continued on next page

Materials Needed for IEF

You will need the following items. Ordering information is on page 57 and recipes are provided on pages 62-63, if you are preparing your own buffers.

- Protein sample
- Deionized water
- IEF markers
- IEF Sample Buffer
- IEF Cathode Buffer
- IEF Anode Buffer

Preparing Sample

The Novex® IEF Sample Buffer (2X) is available from Invitrogen (see page 57 for ordering information).

1. Prepare samples for IEF Gels as described below:

Reagent	Amount
Sample	x µl
Novex® IEF Sample Buffer pH 3-10 or pH 3-7 (2X)	5 µl
Deionized Water	to 5 µl
Total Volume	10 µl

2. Load the sample immediately on the gel. **Do not heat samples for IEF Gels.**

See page 62 for a recipe of the sample buffer, if you are preparing the buffer.

Preparing Running Buffer

Novex® IEF Cathode (10X) and Anode Buffers (50X) are available from Invitrogen (see page 57 for ordering information).

1. Prepare 1000 ml of 1X IEF Anode Buffer using Novex® IEF Anode Buffer (50X) as follows:

Novex® IEF Anode Buffer (50X)	20 ml
Deionized Water	980 ml
Total Volume	1000 ml

2. Mix thoroughly. Use this buffer to fill the Lower Buffer Chamber of the XCell *SureLock*™ Mini-Cell for electrophoresis.
3. Prepare 200 ml of 1X IEF Cathode Buffer using the appropriate Novex® IEF Cathode Buffer pH 3-10 (10X) or pH 3-7 (10X) as follows:

Novex® IEF Cathode Buffer (10X)	20 ml
Deionized Water	180 ml
Total Volume	200 ml

4. Mix thoroughly. Use this buffer to fill the Upper Buffer Chamber of the XCell *SureLock*™ Mini-Cell for electrophoresis.

See page 63 for a recipe of the Novex® IEF Cathode and Anode Buffer, if you are preparing the running buffer.

Continued on next page

Electrophoresis Conditions

Instructions for running the IEF gel using XCell *SureLock*™ Mini-Cell and the electrophoresis conditions are provided on page 30.

Fixing the Gel

We recommend fixing the proteins in the IEF gel using a fixing solution containing 12% TCA or 12% TCA containing 3.5% sulfosalicylic acid for 30 minutes. The fixing step removes the carrier ampholytes from the gel resulting in lower background after staining.

For staining IEF gels, refer to pages 33-38.

For performing second dimension SDS-PAGE, see the next page.

Note

Fixing and staining the IEF gel prior to performing second dimension SDS-PAGE has the following advantages over other methods of storing IEF gels:

- Indefinite storage without loss of resolution
- Easy to manipulate as bands are visible
- Confirms quality of first dimension IEF before proceeding to SDS-PAGE

The SDS in the sample buffer and running buffer for SDS-PAGE strips the stain from proteins and resolubilizes the proteins for migration into the SDS gel.

Materials Needed for 2D SDS-PAGE

You will need the following items. Ordering information for buffers is on page 57.

- Appropriate SDS gel with a 2D-well (Novex® Tris-Glycine or NuPAGE® Novex Gels)
- 20% Ethanol
- Sample Buffer (depending on your gel type)
- Running Buffer (depending on your gel type)
- XCell *SureLock*™ Mini-Cell
- Filter Paper

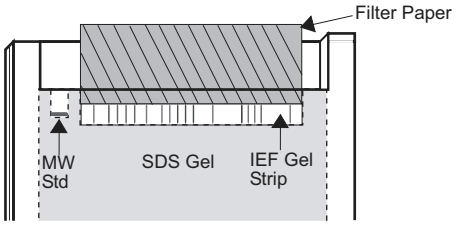
Continued on next page

Performing 2D SDS-PAGE

We recommend using a NuPAGE® or Tris-Glycine SDS Gel with a 2D-well for 2D SDS-PAGE. The length of the 2D-well of a Novex® SDS gel is 6.5 cm.

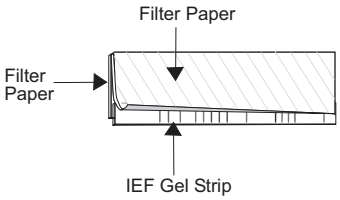
1. After staining and destaining the IEF gel, incubate the IEF gel in 100 ml 20% ethanol for 10 minutes.
2. Cut out the desired lane (strip) from the IEF gel for transfer to a SDS gel.
3. Incubate the strip in 2 ml 2X SDS sample buffer and 0.5 ml ethanol for 3-5 minutes. Aspirate the sample buffer and rinse the strip with the appropriate 1X SDS Running Buffer.
4. Fill the SDS gel cassette with the appropriate 1X SDS Running Buffer.
5. Trim the IEF strip to a length of 5.8-5.9 cm.
6. Transfer the strip into a 1.0 mm or 1.5 mm SDS Gel as described below:
   - **1.0 mm SDS Gel**

     Slide the strip into the gel cassette and into the 2D-well using a gel-loading tip. Avoid trapping any air-bubbles between the gel strip and the surface of the SDS gel. Wet a piece of thick filter paper (5.8 x 4 cm) in 1X SDS Running Buffer and insert the long edge of the paper into the SDS gel so the paper rests on top of the IEF gel strip (see figure below). Push the filter paper down so the IEF gel strip makes contact with the SDS gel. The paper will hold the IEF gel strip in place.



   - **1.5 mm SDS Gel**

     Wet 2 pieces of thin filter paper (5.8 x 4 cm) in 1X SDS Running Buffer. Prepare a sandwich of the filter papers and the IEF gel strip along the long edge of the paper so the strip is sandwiched between the two filter papers with the edge of the strip protruding ~0.5 mm beyond the paper (see figure below). Insert the sandwich into the 2D-well of the SDS gel. Avoid trapping any air-bubbles between the gel strip and the surface of the SDS gel. Push the strip down so it is in contact with the SDS gel



7. Insert gel cassette into the mini-cell, fill the buffer chambers with 1X SDS Running Buffer, load molecular weight markers, and perform SDS-PAGE.
8. After the dye front has moved into the stacking gel (~10 minutes), disconnect the power supply, remove the filter paper, and resume electrophoresis to completion.
9. Proceed to staining the second dimension gel using a method of choice.

ZOOM® Gels

ZOOM® Gels

ZOOM® Gels are 8 x 8 cm, 1.0 mm thick pre-cast polyacrylamide gels cast in a 10 x 10 cm cassette. The ZOOM® Gels are used for 2D analysis of proteins following isoelectric focusing of 7.0 cm IPG strips. ZOOM® Gels contain an IPG well and a molecular weight marker well. The IPG well is designed to accommodate a 7.0 cm IPG strip.

Two types of ZOOM® Gels are available (see page 57 for ordering information)

- NuPAGE® Novex 4-12% Bis-Tris ZOOM® Gel
- Novex® 4-20% Tris-Glycine ZOOM® Gel

Second Dimension Electrophoresis

The second dimension electrophoresis procedure involves reducing and alkylating the proteins focused on your IPG strip in equilibration buffer, loading the strip on your second dimension gel, and performing SDS-PAGE.

Materials Supplied by the User

You will need the following items.

- 4X NuPAGE® LDS Sample Buffer (see page 57 for ordering information)
- NuPAGE® Sample Reducing Agent (see page 57 for ordering information)
- NuPAGE® Novex 4-12% Bis-Tris ZOOM® Gel or Novex® 4-20% Tris-Glycine ZOOM™ Gel (see page 57 for ordering information)
- Appropriate running buffer depending on the type of gel you are using (see page 57 for ordering information)
- 0.5% agarose solution
- Iodoacetamide
- Plastic flexible ruler or thin weighing spatula
- 15 ml conical tubes
- Water bath set at 55°C or 65°C
- XCell *SureLock*™ Mini-Cell (see page 57 for ordering information)
- Protein molecular weight marker (see page 57)

Equilibrating the IPG Strip

1. Dilute 4X NuPAGE® LDS Sample Buffer to 1X with deionized water.
2. Add 500 µl of the NuPAGE® Sample Reducing Agent (10X) to 4.5 ml of the 1X NuPAGE® LDS Sample Buffer from Step 1 in a 15 ml conical tube. Place one IPG strip in this conical tube for equilibration.
3. Incubate for 15 minutes at room temperature. Decant the Reducing Solution.
4. Prepare 125 mM Alkylating Solution by adding 232 mg of fresh iodoacetamide to 10 ml of 1X NuPAGE® LDS Sample Buffer from Step 1.
5. Add 5 ml of Alkylating Solution (from Step 4) to the conical tube containing the IPG strip. Incubate for 15 minutes at room temperature.
6. Decant the Alkylating Solution and proceed to **SDS-PAGE**, next page.

Use the equilibrated IPG strip immediately for second dimension SDS-PAGE.

Continued on next page

SDS-PAGE

A protocol for SDS-PAGE is provided below using ZOOM® Gels with the XCell *SureLock*™ Mini-Cell. You may download the XCell *SureLock*™ Mini-Cell manual from our web site at www.invitrogen.com or contact Technical Service (see page 66). If you are using any other electrophoresis system, refer to the manufacturer's recommendations.

1. Prepare 0.5% agarose solution in the appropriate running buffer and keep it warm (55-65°C) until you are ready to use the agarose solution.
2. Cut the plastic ends of the IPG strip flush with the gel. Do not cut off any portions of the gel.
3. Slide the IPG strip into the ZOOM® Gel well.
4. If the molecular weight marker well is bent, straighten the well using a gel-loading tip.
5. Align the IPG strip properly in the ZOOM® Gel well using a thin plastic ruler or a weighing spatula. Avoid introducing any air bubbles while sliding the strip.
6. Pour ~ 400 µl of 0.5% agarose solution into the ZOOM® Gel well containing the IPG strip. Take care that the agarose solution does not overflow into the molecular weight marker well.
7. Assemble the gel cassette/Buffer Core sandwich as described in the XCell *SureLock*™ Mini-Cell manual. If you are using only one gel, use the Buffer Dam to replace the second gel cassette.

   Note: Do not use the ZOOM® IPGRunner™ Core for electrophoresis of the second dimension gel. You must use the Buffer Core supplied with the XCell *SureLock*™ Mini-Cell.
8. Fill the Lower Buffer Chamber and Upper Buffer Chamber with the appropriate running buffer.
9. Load molecular weight standards in the marker well.
10. Place the XCell *SureLock*™ Mini-Cell lid on the Buffer Core. With the power on the power supply turned off, connect the electrode cords to the power supply [red to (+) jack, black to (-) jack].
11. Perform electrophoresis at 200 V for 40 minutes for NuPAGE® Novex Bis-Tris ZOOM® Gel or at 125 V for 90 minutes for Novex® Tris-Glycine ZOOM® Gel.
12. At the end of electrophoresis, turn off the power and disassemble the gel cassette/Buffer Core sandwich assembly as described in the XCell *SureLock*™ Mini-Cell manual.
13. Proceed to staining the second dimension gel using a method of choice.

TBE Gels

Introduction

Novex® polyacrylamide TBE Gels provide high-resolution analysis of restriction digests and PCR products. The TBE Gels give sharp, intense bands and provide separations of double-strand DNA fragments from 10-3000 base pairs.

Advantages

Using polyacrylamide gels for nucleic acid separation provides the following advantages over agarose gels:

- High resolution and sensitivity
- Lower background staining
- Requires less sample concentration and volume
- Efficient blotting
- Easy to extract DNA from the gel and does not interfere with enzymatic reactions
- Accurate and reproducible results

Materials Supplied by the User

You will need the following items. Ordering information is on page 57 and recipes are provided on pages63-64, if you are preparing your own buffers.

- DNA sample
- Deionized water
- Appropriate DNA markers
- Hi-Density TBE Sample Buffer
- TBE Running Buffer

Preparing Samples

The Novex® Hi-Density TBE Sample Buffer (5X) is available from Invitrogen (see page 57 for ordering information).

1. Prepare samples for TBE gels as described below:

Reagent	Amount
Sample	x µl
Novex® Hi-Density TBE Sample Buffer (5X)	2 µl
Deionized Water	to 8 µl
Total Volume	10 µl

2. Load the samples immediately on the gel.

See page 64 for a recipe of the sample buffer, if you are preparing the sample buffer.

Continued on next page

Preparing Running Buffer

Novex® TBE Running Buffer (5X) is available from Invitrogen (see page 57 for ordering information).

1. Prepare 1000 ml of 1X TBE Running Buffer using Novex® TBE Buffer (5X) as follows:

Novex® TBE Running Buffer (5X)	200 ml
Deionized Water	800 ml
Total Volume	1000 ml

2. Mix thoroughly. Use this buffer to fill the Upper and Lower Buffer Chamber of the XCell *SureLock*™ Mini-Cell for electrophoresis.

See page 63 for a recipe of the Novex® TBE Running Buffer, if you are preparing the running buffer.

Electrophoresis Conditions

Instructions for running the TBE gel using XCell *SureLock*™ Mini-Cell and the electrophoresis conditions are provided on page 30.

Migration of the Dye Fronts

The size of the DNA fragments visualized at the dye fronts of the different TBE Gels is shown in the table below.

Gel Type	Dye Front*	
	Bromophenol Blue (dark blue)	**Xylene Cyanol (blue green)**
6% TBE Gel	65 bp	250 bp
8% TBE Gel	25 bp	220 bp
10% TBE Gel	35 bp	120 bp
20% TBE Gel	15 bp	50 bp
4-12% TBE Gel	35 bp	400 bp
4-20% TBE Gel	25 bp	300 bp

*accuracy is ± 5 bp

TBE-Urea Gels

Introduction

Novex® denaturing polyacrylamide TBE-Urea Gels provide high resolution of short single-strand oligonucleotides. The TBE-Urea Gels provide excellent resolution for fast size and purity confirmations of DNA or RNA oligos from 20-600 bases.

The TBE-Urea Gels contain 7 M urea for maximum denaturation.

Materials Supplied by the User

You will need the following items. Ordering information is provided on page 57 and recipes for buffers are on page 64, if you are preparing your own buffers.

- DNA or RNA sample
- Deionized water
- Appropriate DNA or RNA markers
- TBE-Urea Sample Buffer
- Prep TBE-Urea Sample Buffer for preparative gel electrophoresis
- TBE Running Buffer

To obtain optimal results with TBE-Urea Gels, observe the following recommendations:

- Load only about 1/10 sample as used on large gels or agarose gels. Dilute your standards and samples to ~ 0.01 OD (0.2 µg/band)
- Use RNase-free ultrapure water
- Prior to loading samples, flush wells several times with 1X TBE Running Buffer to remove urea
- Load samples quickly and avoid allowing the gel to stand for long periods of time after loading to prevent diffusion
- Use Prep TBE-Urea Sample Buffer for preparative gel electrophoresis as this buffer does not contain any marker dyes
- Wear gloves and use dedicated equipment to prevent contamination
- Heat samples at 70°C for 3 minutes to denature the samples and keep the samples on ice to prevent renaturation
- Avoid using buffers with formamide on TBE-Urea polyacylamide gels as it will result in fuzzy bands

Continued on next page

TBE-Urea Gels, Continued

Preparing Samples

The Novex® TBE-Urea Sample Buffer (2X) is available from Invitrogen (see page 57) for ordering information).

1. Prepare samples for TBE-Urea Gels as described below:

Reagent	Amount
Sample	x µl
Novex® TBE-Urea Sample Buffer (2X)	5 µl
Deionized Water	to 5 µl
Total Volume	10 µl

2. Flush wells of the gel several times with 1X TBE Running Buffer (see below) to remove urea from the wells prior to loading samples to obtain sharp bands.
3. Load the samples immediately on the gel.

See page 64 for a recipe of the sample buffer, if you are preparing the sample buffer.

Preparing Running Buffer

Novex® TBE Running Buffer (5X) is available from Invitrogen (see page 57 for ordering information).

1. Prepare 1000 ml of 1X TBE Running Buffer using Novex® TBE Buffer (5X) as follows:

Novex® TBE Running Buffer (5X)	200 ml
Deionized Water	800 ml
Total Volume	1000 ml

2. Mix thoroughly. Use this buffer to fill the Upper and Lower Buffer Chamber of the XCell *SureLock*™ Mini-Cell for electrophoresis.

See page 63 for a recipe of the Novex® TBE Running Buffer, if you are preparing the running buffer.

Electrophoresis Conditions

Instructions for running the TBE-Urea gel using XCell *SureLock*™ Mini-Cell and the electrophoresis conditions are provided on page 30.

Migration of the Dye Fronts

The size of the single-strand DNA fragments visualized at the dye fronts of the different TBE-Urea Gels is shown in the table below.

Gel Type	Dye Front*	
	Bromophenol Blue (dark blue)	Xylene Cyanol (light blue)
6% TBE-Urea Gel	25 bases	110 bases
10% TBE-Urea Gel	20 bases	55 bases
15% TBE-Urea Gel	10 bases	40 bases

*accuracy is ± 5 bases

DNA Retardation Gels

Introduction

Novex® DNA Retardation Gels consist of 6% polyacrylamide prepared with 1/2X TBE as the gel buffer. The 6% gel provides good resolution of fragments in the range of 60-2500 bp used for DNA retardation assays. The 1/2X TBE buffer is sufficient for good electrophoretic separation yet low enough to promote DNA/ protein interactions.

Gel-Shift Assay

The gel shift assay is based on the fact that the movement of a DNA molecule through a non-denaturing polyacrylamide gel is hindered when bound to a protein molecule (Revzin, 1989). This technique is used to characterize DNA/protein complexes. Detection is performed with ethidium bromide staining of DNA or, for greater sensitivity, with radiolabeling the DNA or protein.

Materials Supplied by the User

You will need the following items:

- DNA sample
- Deionized water
- Hi-Density TBE Sample Buffer (see page 57 for ordering information or page 64 for a recipe)
- TBE Running Buffer (see page 57 for ordering information or page 63 for a recipe)

Note

Specific buffer conditions may be required during incubation of the protein and DNA target sequence in order to minimize non-specific DNA/protein interactions for certain samples.

If salt concentration is low (0.1 M or less), the samples can usually be loaded in the incubation buffer after adding about 3-5% glycerol and a small amount of bromophenol blue tracking dye.

Preparing Samples

The Novex® Hi-Density TBE Sample Buffer (5X) is available from Invitrogen (see page 57 for ordering information).

1. Prepare samples for DNA Retardation Gels as described below:

Reagent	Amount
Sample	x µl
Novex® Hi-Density TBE Sample Buffer (5X)	1 µl
Deionized Water	to 9 µl
Total Volume	10 µl

2. Load the samples immediately on the gel.

See page 64 for a recipe of the sample buffer, if you are preparing the sample buffer.

Continued on next page

Preparing Running Buffer

Novex® TBE Running Buffer (5X) is available from Invitrogen (see page 57 for ordering information).

1. Prepare 1000 ml of 1/2X (0.5X) TBE Running Buffer using Novex® TBE Buffer (5X) as follows:

Novex® TBE Running Buffer (5X)	100 ml
Deionized Water	900 ml
Total Volume	1000 ml

2. Mix thoroughly. Use this buffer to fill the Upper and Lower Buffer Chamber of the XCell *SureLock*™ Mini-Cell for electrophoresis.

See page 63 for a recipe of the Novex® TBE Running Buffer, if you are preparing the running buffer.

Electrophoresis Conditions

Instructions for running the DNA Retardation gel using XCell *SureLock*™ Mini-Cell and the electrophoresis conditions are provided on page 30.

Electrophoresis of Novex® Pre-Cast Gels

Introduction

Instructions are provided below for electrophoresis of the Novex® Pre-Cast Gels using the XCell *SureLock*™ Mini-Cell. For more information on the XCell *SureLock*™ Mini-Cell, refer to the manual (IM-9003). This manual is available on our Web site at www.invitrogen.com or contact Technical Service (see page 66).

For information on sample and buffer preparation, see pages 10-28.

If you are using any other mini-cell for electrophoresis, refer to the manufacturer's recommendations.

Protocol using XCell *SureLock*™ Mini-Cell

Wear gloves and safety glasses when handling gels.

XCell *SureLock*™ Mini-Cell requires 200 ml for the Upper Buffer Chamber and 600 ml for the Lower Buffer Chamber.

1. Remove the Novex® Pre-Cast Gel from the pouch.
2. Rinse the gel cassette with deionized water. Peel off the tape from the bottom of the cassette.
3. In one smooth motion, gently pull the comb out of the cassette.
4. Rinse the sample wells with the appropriate 1X SDS Running Buffer. Invert the gel and shake the gel to remove the buffer. Repeat two more times.
5. Orient the two gels in the Mini-Cell such that the notched "well" side of the cassette faces inwards toward the Buffer Core. Seat the gels on the bottom of the Mini-Cell and lock into place with the Gel Tension Wedge. Refer to the XCell *SureLock*™ Mini-Cell manual (IM-9003) for detailed instructions.

Note: If you are using only one gel, the plastic Buffer Dam replaces the second gel cassette.

6. Fill the Upper Buffer Chamber with a small amount of the running buffer to check for tightness of seal. If you detect a leak from Upper to the Lower Buffer Chamber, discard the buffer, reseal the chamber, and refill.
7. Once the seal is tight, fill the Upper Buffer Chamber (inner) with the appropriate 1X running buffer. The buffer level must exceed the level of the wells.
8. Load an appropriate volume of sample at the desired protein concentration onto the gel (see page 6 for recommended loading volumes).
9. Load appropriate protein molecular weight markers (see page 57 for ordering information).
10. Fill the Lower Buffer Chamber with 600 ml of the appropriate 1X running buffer.
11. Place the XCell *SureLock*™ Mini-Cell lid on the Buffer Core. With the power on the power supply turned off, connect the electrode cords to the power supply [red to (+) jack, black to (-) jack].
12. See next page for **Electrophoresis Conditions**.

Continued on next page

Electrophoresis of Novex® Pre-Cast Gels, Continued

Electrophoresis Conditions

Run your gels according to the following protocol:

Gel Type	Voltage	Expected Current*	Run Time
Tris-Glycine Gels (SDS-PAGE)	125 V constant	Start: 30-40mA End: 8-12 mA	90 minutes (dependent on gel type) Run the gel until the bromophenol blue tracking dye reaches the bottom of the gel.
Tris-Glycine Gels (Native-PAGE)	125 V constant	Start: 6-12 mA End: 3-6 mA	1-12 hours
Tricine Gels	125 V constant	Start: 80 mA End: 40 mA	90 minutes (dependent on gel type) Run the gel until the phenol red tracking dye reaches the bottom of the gel.
Zymogram Gels	125 V constant	Start: 30-40 mA End: 8-12 mA	90 minutes (dependent on gel type) Run the gel until the bromophenol blue tracking dye reaches the bottom of the gel.
IEF Gels	100 V constant-1 hour 200 V constant-1 hour 500 V constant-30 min	Start: 5 mA End: 6 mA	2.5 hours
TBE Gels	200 V constant**	Start: 10-18 mA End: 4-6 mA	30-90 minutes (dependent on gel type) Run the gel until the bromophenol blue tracking dye reaches the bottom of the gel.
6% TBE-Urea Gels	180 V constant**	Start: 19 mA End: 14 mA	50 minutes Run the gel until the bromophenol blue tracking dye reaches the bottom of the gel.
10% TBE-Urea Gels	180 V constant**	Start: 15 mA End: 8 mA	60 minutes Run the gel until the bromophenol blue tracking dye reaches the bottom of the gel.
15 % TBE-Urea Gels	180 V constant**	Start: 13 mA End: 6 mA	75 minutes Run the gel until the bromophenol blue tracking dye reaches the bottom of the gel.
DNA Retardation Gels	100 V constant	Start: 12-15 mA End: 6-15 mA	90 minutes Run the gel until the bromophenol blue tracking dye reaches the bottom of the gel.

*Expected start and end current values are stated for single gels.
**Voltages up to 250 V may be used to reduce the run time.

Continued on next page

Electrophoresis of Novex® Pre-Cast Gels, Continued

Removing the Gel after Electrophoresis

1. After electrophoresis is complete, shut off the power, disconnect electrodes, and remove gel(s) from the XCell *SureLock*™ Mini-Cell.
2. Separate each of the three bonded sides of the cassette by inserting the Gel Knife into the gap between the cassette's two plates. The notched ("well") side of the cassette should face up.
3. Push down gently on the knife handle to separate the plates. Repeat on each side of the cassette until the plates are completely separated.

   Caution: Use caution while inserting the gel knife between the two plates to avoid excessive pressure towards the gel.
4. Carefully remove and discard the top plate, allowing the gel to remain on the bottom (slotted) plate.
5. If blotting, proceed to page 46 without removing the gel from the bottom plate.
6. If staining, remove the gel from the plate by one of the methods:
   - Use the sharp edge of the gel knife to remove the bottom lip of the gel. The gel knife should be at a 90° angle, perpendicular to the gel and the slotted half of the cassette. Push down on the knife, and then repeat the motion across the gel to cut off the entire lip. Hold the plate and gel over a container with the gel facing downward and use the knife to carefully loosen one lower corner of the gel and allow the gel to peel away from the plate.
   - Hold the plate and gel over a container with the gel facing downward. Gently push the gel knife through the slot in the cassette, until the gel peels away from the plate. Cut the lip off of the gel after fixing, staining, but before drying.
7. Fix and stain the gel as described on pages 33-42. For developing the Zymogram gel for enzyme activity, see page 17. For fixing IEF gels, see page 20.

Silver Staining

Introduction

Instructions are provided below for silver staining Novex® Gels using the SilverQuest™ Silver Staining Kit and the SilverXpress® Silver Staining Kit (see page 57 for ordering information).

If you are using any other silver staining kit, follow the manufacturer's recommendations.

Molecular Weight Calibration

Guidelines and apparent molecular weight values for Novex® protein molecular weight standards are provided on page 52.

Materials Supplied by the User

You will need following items for silver staining. Ordering information is provided on page 57.

- Staining container
- Rotary Shaker
- Ultrapure water (>18 megohm/cm resistance recommended)
- Teflon coated stir bars
- Disposable 10 ml pipettes
- Clean glass bottles for reagent preparation
- Graduated glass cylinders
- Protein molecular weight markers (Mark 12™ Unstained Standard, recommended)

For SilverQuest™ Staining:

- SilverQuest™ Silver Staining Kit
- 30% ethanol (made with ultrapure water)
- 100% ethanol
- Fixative (40% ethanol, 10% acetic acid, made with ultrapure water)

For SilverXpress® Staining:

- SilverXpress® Silver Staining Kit
- Methanol
- Acetic acid
- Sulfosalicylic acid
- Trichloroacetic acid (TCA)

Continued on next page

For optimal silver staining results, follow these guidelines:

- Be sure to wear rubber gloves that have been rinsed with deionized water while handling gels
- Use clean containers and designate these containers for silver staining purposes only
- Make sure the size of the container permits free movement of the gel during shaking and complete immersion in solution while staining
- Do not touch the gel with bare hands or metal objects and do not put pressure on gels while handling or changing solutions
- Use teflon coated stir bars and clean glass containers to prepare reagents
- Avoid cross contamination of kit reagents
- Use freshly made solutions

Preparing Solutions for SilverQuest™ Silver Staining

Use the reagents provided in the SilverQuest™ Silver Staining Kit to prepare the following solutions for staining:

- Sensitizing solution

Ethanol	30 ml
Sensitizer	10 ml
Ultrapure water	to 100 ml

- Staining solution

Stainer	1 ml
Ultrapure water	to 100 ml

- Developing solution

Developer	10 ml
Developer enhancer	1 drop
Ultrapure water	to 100 ml

Note: You may prepare all solutions immediately before starting the staining protocol or prepare them as you proceed to the next step.

Continued on next page

SilverQuest™ Microwave Silver Staining Protocol

The Fast Staining protocol (using a microwave oven) for silver staining Novex® Gels using SilverQuest™ Silver Staining Kit is described below. For the Basic Protocol and more details on the staining procedure, refer to the SilverQuest™ Silver Staining Kit Manual (IM-6070). This manual is available on our Web site at www.invitrogen.com or contact Technical Service (see page 66).

For use with an 8 x 8 cm Novex® Gel, 1.0 mm thick. Use 100 ml of each solution per gel.

Note: You may have to optimize the staining protocol, if the dimensions of your gel are not the same as mentioned above.

Caution: Use caution while performing the Fast Staining Protocol using a microwave oven. Do not overheat the staining solutions.

1. After electrophoresis, place the gel in a clean microwaveable staining tray of the appropriate size. Rinse the gel briefly with ultrapure water.
2. Place the gel in 100 ml of fixative and microwave at high power (700 watts) for 30 seconds. Remove the gel from the microwave and gently agitate it for 5 minutes at room temperature. Decant the fixative.
3. Wash the gel with 100 ml of 30% ethanol in a microwave at high power for 30 seconds. Remove the gel from the microwave and gently agitate it for 5 minutes at room temperature on a rotary shaker. Decant the ethanol.
4. Add 100 ml of Sensitizing solution to the washed gel. Microwave at high power for 30 seconds. Remove the gel from the microwave and place it on a rotary shaker for 2 minutes at room temperature. Decant the Sensitizing solution.
5. Wash the gel twice in 100 ml ultrapure water. Microwave at high power for 30 seconds. At each wash step, remove the gel from the microwave and gently agitate it for 2 minutes at room temperature.
6. Place the gel in 100 ml of Staining solution. Microwave at high power for 30 seconds. Remove the gel from the microwave and gently agitate it for 5 minutes at room temperature.
7. Decant the Staining solution and wash the gel with 100 ml of ultrapure water for 20-60 seconds. Do not wash the gel for more than a minute.
8. Place the gel in 100 ml of Developing solution and incubate for 5 minutes at room temperature with gentle agitation on a rotary shaker. **Do not microwave**.
9. Once the desired band intensity is achieved, immediately add 10 ml of Stopper directly to the gel still immersed in Developing solution and gently agitate the gel for 10 minutes. The color changes from pink to clear indicating that the end of development.
10. Wash the gel with 100 ml of ultrapure water for 10 minutes. For gel drying, see page 43.

If you need to destain the gel for mass spectrometry analysis, see the SilverQuest™ Silver Staining Kit Manual (IM-6070).

Continued on next page

Preparing Solutions for SilverXpress® Silver Staining

Prepare the reagents as described below. If you are staining two gels, double the reagent volumes. **Note:** The final volumes of solutions containing methanol and water reflect a volume shrinkage which occurs when these two reagents are mixed. Do not adjust volumes of components or final volume.

- **Fixing solution** for Tris-Glycine and Tricine Gels

Methanol	100 ml
Acetic Acid	20 ml
Ultrapure water	90 ml

- **Fixing solution** for TBE, TBE-Urea Gels

Sulphosalicylic acid	7 g
TCA	24 g
Ultrapure water	200 ml

- **Sensitizing solution**

Methanol	100 ml
Sensitizer	5 ml
Ultrapure water	105 ml

- **Staining solution**

Stainer A	5 ml
Stainer B	5 ml
Ultrapure water	90 ml

- **Developing Solution**

Developer	5 ml
Ultrapure water	95 ml

Continued on next page

SilverXpress® Silver Staining Protocol

The following staining procedure is for 1 mm Novex® Gel. If you are using 1.5 mm Novex® Gel, double the incubation time.

For gel drying, see page 43.

Note: Gels may be stored in the second Sensitizing Solution overnight, if desired.

Step	Solution	Vol/Gel	Gel Type			
			Tris-Glycine	Tricine	TBE/TBE-Urea	IEF
1A	Fix the gel in Fixing Solution.	200 ml	10 minutes	10 minutes	10 minutes	10 minutes
1B		N/A	N/A	N/A	N/A	10 minutes
2A	Decant the Fixing Solution and incubate the gel in two changes of Sensitizing Solution.	100 ml	10 minutes	30 minutes	10 minutes	30 minutes
2B		100 ml	10 minutes	30 minutes	10 minutes	30 minutes
3A	Decant the Sensitizing Solution and rinse the gel twice with ultrapure water.	200 ml	5 minutes	5 minutes	5 minutes	5 minutes
3B		200 ml	5 minutes	5 minutes	5 minutes	5 minutes
4	Incubate the gel in Staining Solution.	100 ml	15 minutes	15 minutes	30 minutes	15 minutes
5A	Decant the Staining Solution and rinse the gel twice with ultrapure water.	200 ml	5 minutes	5 minutes	5 minutes	5 minutes
5B		200 ml	5 minutes	5 minutes	5 minutes	5 minutes
6	Incubate the gel in Developing Solution.	100 ml	3-15 minutes	3-15 minutes	3-15 minutes	3-15 minutes
7	Add the Stopping Solution directly to the gel when the desired staining intensity is reached.	5 ml	10 minutes	10 minutes	10 minutes	10 minutes
8A	Decant the Stopping Solution and wash the gel three times in ultrapure water.	200 ml	10 minutes	10 minutes	10 minutes	10 minutes
8B		200 ml	10 minutes	10 minutes	10 minutes	10 minutes
8C		200 ml	10 minutes	10 minutes	10 minutes	10 minutes

Coomassie® Staining

Introduction

Instructions are provided below for coomassie® staining Tris-Glycine, Zymogram, IEF, and Tricine Gels using the SimplyBlue™ SafeStain, Colloidal Blue Staining Kit (see page 57 for ordering information), and Coomassie® R-250.

If you are using any other coomassie® staining kit, follow the manufacturer's recommendations.

Note

If you are staining low molecular weight peptides (< 2.5 kDa), we recommend fixing the gel in 5% glutaraldehyde and 50% methanol for one hour and then follow the instructions in the Colloidal Blue Staining Kit Manual (IM-6025) for small peptides.

Molecular Weight Calibration

Guidelines and apparent molecular weight values for Novex® protein molecular weight standards are provided on page 52.

Materials Supplied by the User

You will need the following items for staining. Ordering information is provided on page 57.

- SimplyBlue™ SafeStain
- Colloidal Blue Staining Kit
- Staining container
- Deionized water
- Orbital Shaker
- 0.1% Coomassie® R-250 in 40% ethanol and 10% acetic acid (if using Coomassie® R-250 Staining, see page 41)
- Destaining Solution consisting of 10% ethanol and 7.5% acetic acid (if using Coomassie® R-250 Staining, see page 41)
- Methanol

Continued on next page

SimplyBlue™ SafeStain Protocol

The Basic Protocol for staining Novex® Gels with SimplyBlue™ SafeStain is provided below. For the Microwave Protocol and staining large format gels, refer to the SimplyBlue™ SafeStain Manual (IM-6050). This manual is available on our Web site at www.invitrogen.com or contact Technical Service (see page 66).

For general use with 1.0 mm and 1.5 mm Tris-Glycine gels, 1.0 mm Tricine, Zymogram, and IEF mini-gels (8 x 8 cm).

After electrophoresis follow the instructions below. Be sure the mini-gel moves freely in water or stain to facilitate diffusion during all steps.

Note: Stain the Zymogram gel with SimplyBlue™ SafeStain after renaturing and developing the gel for enzyme activity.

1. Fix the IEF gel in 100 ml 12% TCA for 15 minutes. The fixing step is not required for Tris-Glycine, Tricine, and Zymogram Gels.
2. **Rinse** the mini-gel 3 times for 5 minutes with 100 ml deionized water to remove SDS and buffer salts, which interfere with binding of the dye to the protein. Discard each rinse.
3. **Stain** the mini-gel with enough SimplyBlue™ SafeStain (20-100 ml) to cover the gel. Stain for 1 hour at room temperature with gentle shaking. Bands will begin to develop within minutes. After incubation, discard the stain. Stain cannot be re-used.

Note: Gel can be stained for up to 3 hours, but after 3 hours, sensitivity will decrease. If you need to leave the gel overnight in the stain, add 2 ml of 20% NaCl (w/v) in water for every 20 ml of stain. This procedure will not affect sensitivity.

4. **Wash** the mini-gel with 100 ml of water for 1-3 hours. The gel can be left in the water for several days without loss of sensitivity. There is a small amount of dye in the water that is in equilibrium with the dye bound to the protein, so proteins will remain blue.
5. To obtain the clearest background for photography, perform a second 1 hour wash with 100 ml water.

Note: Sensitivity will now decrease if the gel is allowed to stay in the water more than 1 day. Reduction of free dye in the water favors dissociation of the dye from the protein. If you need to store the gel in water for a few days, add 20 ml of 20% NaCl.

6. For gel drying, see page 43.

Continued on next page

Colloidal Blue Staining Kit Protocol

A brief staining protocol for staining Novex® Gels with the Colloidal Blue Staining Kit (see page 57 for ordering information) is provided below. For more details on the staining procedure, refer to the Manual (IM-6025). This manual is available on our Web site at www.invitrogen.com or contact Technical Service (see page 66).

1. Fix the IEF Gel in fixing solution as described on page 20. This step is not required for Tris-Glycine, Tricine, and Zymogram Gels.
2. Prepare staining solution for a single gel as described in the table below. For two gels, double the volume of reagents used for staining. Be sure to shake Stainer B prior to making the solution.

Solutions	Tris-Glycine, Tricine, and Zymogram Gel	IEF Gel
Deionized Water	55 ml	58 ml
Methanol	20 ml	20 ml
Stainer B	5 ml	2 ml
Stainer A	20 ml	20 ml

3. Incubate the gel in this staining solution as follows at room temperature with gentle shaking:
   - Tris-Glycine, Tricine, and Zymogram Gels for a minimum of 3 hours and a maximum of 12 hours .
   - IEF Gels for 30 minutes.
4. Decant staining solution and add a minimum of 200 ml of deionized water per gel to the staining container. Gently shake gel in water for at least 7 hours. Gel will have a clear background after 7 hours in water.
5. For gel drying, see page 43.

Note: Novex® Gels can be left in deionized water for up to 3 days without significant change in band intensity and background clarity.

For long-term storage (over 3 days), keep the gel in a 20% ammonium sulfate solution at 4°C.

Coomassie® Staining, Continued

Coomassie® R-250 Staining Protocol

The coomassie® staining protocol described below is recommended for staining Novex® Gels. You may use any Coomassie® staining protocol of choice.

1. Prepare the staining solution containing 0.1% Coomassie® R-250 in 40% ethanol, 10% acetic acid.
2. After electrophoresis, incubate 1 or 2 gels in a staining container containing 100 ml Coomassie® Blue R-250 staining solution.

   Caution: Use caution while performing the following steps using a microwave oven. Do not overheat the staining solutions.
3. Loosely cover the staining container and heat in a microwave oven at full power for 1 minute. To prevent hazardous, flammable vapors from forming, do not allow the solution to boil.
4. Remove the staining container from the microwave oven and gently shake the gel for 15 minutes at room temperature on an orbital shaker.
5. Decant the stain and rinse the gel once with deionized water.
6. Prepare a destain solution containing 10% ethanol and 7.5% acetic acid.
7. Place one or two stained gels in a staining container containing the 100 ml destain solution.
8. Loosely cover the staining container and heat in a microwave oven at full power for 1 minute.
9. Gently shake the gel at room temperature on an orbital shaker until the desired background is achieved.
10. For gel drying, see page 43.

Ethidium Bromide Staining

Introduction

A brief protocol is provided below for staining TBE and TBE-Urea Gels with ethidium bromide. You may use any other staining method of choice.

Procedure

Caution: Ethidium bromide is a powerful mutagen and is moderately toxic. Wear gloves and protective clothing when handling ethidium bromide solutions.

1. Prepare 2 µg/ml solution of ethidium bromide in ultrapure water.
2. Incubate the gel in the ethidium bromide solution for 20 minutes.
3. Destain the gel by rinsing the gel three times with ultrapure water for 10 minutes,
4. Remove the gel from the cassette using a Gel Knife to visualize bands under UV light.

Note

Ethidium bromide staining of polyacrylamide gels requires at least 10 ng of DNA for detection due to the quenching of the fluorescence by polyacrylamide. Silver staining using SilverXpress® Silver Staining Kit (see page 37) will provide greater detection sensitivity.

Gel Drying

Introduction

Gels can be dried using passive evaporation (air-drying) or vacuum drying. Vacuum drying is faster than passive air-drying methods but often results in cracked gels due to the speed of dehydration.

We recommend drying Novex® Pre-Cast gels using passive air-drying methods such as DryEase® Mini-Gel Drying System (see below). If certain applications require drying the gel using vacuum drying, follow the recommendations on page 45 to minimize cracking of vacuum dried gels.

Materials Supplied by the User

You will need the following items:

- DryEase® Mini-Gel Drying System (see page 57 for ordering information)
- Gel-Dry™ Drying Solution (or prepare your own gel drying solution containing 30% methanol and 5% glycerol)
- StainEase® Gel Staining Tray (see page 57 for ordering information) or a round container

DryEase® Mini-Gel Drying System

Silver stained and Coomassie® stained Novex® Gels can be dried by vacuum drying or by air-drying. We recommend using the DryEase® Mini-Gel Drying System to air-dry the gel.

A brief gel drying protocol using the DryEase® Mini-Gel Drying System is provided below. For more details on this system, refer to the DryEase® Mini-Gel Drying System manual (IM-2380). This manual is available for downloading from our Web site at www.invitrogen.com or contact Technical Service (see page 66).

1. After all staining and destaining steps are complete, wash the destained gel(s) three times for two minutes each time in deionized water (50 ml per mini-gel) on a rotary shaker.
2. Decant the water and add fresh Gel-Dry™ Drying Solution (35 ml per mini-gel).
3. Equilibrate the gel in the Gel-Dry™ Drying Solution by shaking the gel for 15-20 minutes in the StainEase® Gel Staining Tray or in a round container. **Note**: Do not equilibrate gels stained with Coomassie® G-250 in the Gel-Dry™ Drying Solution for more than 5 minutes to avoid losing band intensity.
4. Cut any rough edges off the gel (including the wells and the gel foot) using the Gel Knife or a razor blade.
5. Remove 2 pieces (per gel) of cellophane from the package.
6. Immerse one sheet at a time in the Gel-Dry™ Drying Solution. Allow 10 seconds for complete wetting before adding additional sheets. Do not soak the cellophane for more than 2 minutes.

Continued on next page

DryEase® Mini-Gel Drying System, continued

7. Place one side of the DryEase® Gel Drying Frame with the corner pin facing up, on the DryEase® Gel Drying Base.
8. Center a piece of pre-wetted cellophane from Step 5 over the base/frame combination, so the cellophane lays over the inner edge of the frame.
9. Lay the gel on the center of the cellophane sheet making sure no bubbles are trapped between the gel and the cellophane. Add some Gel-Dry™ Drying Solution to the surface of the cellophane, if necessary.
10. Carefully lay the second sheet of cellophane over the gel so that no bubbles are trapped between the cellophane and the gel. Add some Gel-Dry™ Drying Solution if necessary. Gently smooth out any wrinkles in the assembly with a gloved hand.
11. Align the remaining frame so that its corner pins fit into the appropriate holes on the bottom frame. Push the plastic clamps onto the four edges of the frames.
12. Lift the frame assembly from the DryEase® Gel Drying Base and pour off the excess solution from the base.
13. Stand the gel dryer assembly upright on a bench top. Be careful to avoid drafts as they can cause an uneven rate of dying which leads to cracking. Drying will take between 12–36 hours depending on humidity and gel thickness.
14. When the cellophane is dry to touch, remove the gel/cellophane sandwich from the drying frame. Trim off the excess cellophane.
15. Press the dried gel(s) between the pages of a notebook under light pressure for approximately 2 days. Gels will then remain flat for scanning, photography, display, and overhead projection.

Continued on next page

Vacuum Drying

General guidelines are provided below to minimize cracking during vacuum drying of gels. For detailed instructions on vacuum drying, follow the manufacturer's recommendations.

Handle Gels with Care:

Remove the gel from the cassette without breaking or tearing the edges. Small nicks or tears can act as a starting point for cracking. Remove the gel wells and foot off the bottom of the gel with a Gel Knife or a razor blade as described on page 32. Use the StainEase® Staining Tray for staining and destaining gels. This tray is designed to facilitate the solution changing process without handling of gels.

Use a Gel Drying Solution:

We recommend equilibrating the gel in a gel drying solution such as Gel-Dry™ Gel Drying Solution for 10-30 minutes at room temperature with gentle shaking on an orbital shaker before drying the gel. Gel-Dry™ Gel Drying Solution (see page 57 for ordering information) contains a proprietary non-glycerol component to effectively regulate the rate of drying and prevent cracking. The gel drying solutions do not interfere with autoradiography.

To prepare your own gel drying solution, prepare a solution containing 30% methanol and 5% glycerol.

Note: Do not incubate gels stained with Coomassie® G-250 in gel drying solution for more than 5 minutes as the bands may fade.

Remove Air Bubbles:

Remove any air bubbles that may be trapped between the paper, gel, and plastic wrap by rolling a small glass pipette over the gel. Use additional gel drying solution to help remove the air bubbles.

Use Proper Gel Dryer Set-up:

Place gel on the gel dryer with the plastic wrap facing up. Use a proper working condition vacuum pump and make sure a tight seal is formed by the vacuum. Dry gels using drying conditions set for polyacrylamide gels which require increasing the temperature to a set value and holding this temperature throughout the drying cycle. The recommended conditions for mini-gels are 80°C for 2 hours.

Ensure Gel is Completely Dry:

The gel will crack if the vacuum seal of the heated gel dryer is broken prior to complete drying of the gel. To ensure the gel is completely dried before releasing the vacuum seal, follow these tips :

- Check the temperature of the gel

  The temperature of the dried gel should be the same as the temperature of the surrounding gel drying surface. If the temperature of the dried gel is cooler, then the gel is not completely dried.

- Check for moisture in the tubing connecting the gel dryer to the vacuum pump

  The gel is not completely dried if there is any residual moisture in the tubing and additional drying time is required.

Blotting Novex® Pre-Cast Gels

Introduction

Instructions are provided below for blotting Novex® Pre-Cast Gels using the XCell II™ Blot Module (see page 57 for ordering information). For more information on the XCell II™ Blot Module, refer to the manual (IM-9051) available at www.invitrogen.com or contact Technical Service (see page 66).

If you are using any other blotting apparatus, follow the manufacturer's recommendations.

Power Considerations for Blotting

During blotting, the distance traveled (gel thickness) between the electrodes is much lower than during electrophoresis requiring lower voltage and lower field strength (volts/distance). However, the cross sectional area of current flow is much greater requiring higher current.

Blotting power requirements depend on field strength (electrode size) and conductivity of transfer buffer. The higher the field strength and conductivity of the buffer, the higher is the current requirement (the current decreases during the run as the ions in the buffer polarize). It is important to use a power supply capable of accommodating the initial high current requirement.

Materials Supplied by the User

- Blotting membranes (see page 57 for ordering information)
- Filter paper (not needed if using Novex® pre-cut membrane/filter paper sandwiches)
- Methanol (if using PVDF membranes)
- XCell II™ Blot Module
- Appropriate Transfer Buffer (see page 57 for ordering information)
- Deionized water

Preparing Transfer Buffer

For blotting Tris-Glycine, Tricine, and IEF Gels

We recommend using the Tris-Glycine Transfer Buffer (see page 57 for ordering information). An alternate transfer protocol for IEF gels is provided on page 51.

If you are performing protein sequencing, alternate transfer buffers are listed on the next page.

Prepare 1000 ml of 1X Tris-Glycine Transfer Buffer using the Tris-Glycine Transfer Buffer (25X) as follows:

Tris-Glycine Transfer Buffer (25X)	40 ml
Methanol	200 ml
Deionized Water	760 ml
Total Volume	1000 ml

See page 60 for a recipe of the Tris-Glycine Transfer Buffer, if you are preparing your own transfer buffer.

For blotting TBE and TBE-Urea Gels

Dilute the 5X TBE Running Buffer to 0.5X with deionized water. See page 63 for a recipe of the TBE Running Buffer, if you are preparing your own transfer buffer.

Continued on next page

Blotting Novex® Pre-Cast Gels, Continued

Alternate Transfer Buffers

The Tris-Glycine Transfer Buffer will interfere with protein sequencing. If you are performing protein sequencing, use the NuPAGE® Transfer Buffer or the 0.5X TBE Running Buffer to perform blotting.

The NuPAGE® Transfer Buffer protects against modification of the amino acid side chains and is compatible with N-terminal protein sequencing using Edman degradation.

Preparing Blotting Pads

Use about 700 ml of 1X Transfer Buffer to soak the pads until saturated. Remove the air bubbles by squeezing the pads while they are submerged in buffer. Removing the air bubbles is essential as they can block the transfer of biomolecules if they are not removed.

Preparing Transfer Membrane and Filter

Cut selected transfer membrane and filter paper to the dimensions of the gel or use Novex® pre-cut membrane/filter paper sandwiches (see page 57 for ordering information.

- **PVDF membrane**—Pre-wet PVDF membrane for 30 seconds in methanol, ethanol, or isopropanol. Briefly rinse in deionized water, then place in a shallow dish with 50 ml of 1X Transfer Buffer for several minutes.
- **Nitrocellulose**—Place the membrane directly into a shallow dish containing 50 ml of 1X Transfer Buffer for several minutes.
- **Filter paper**—Soak the filter paper briefly in 1X Transfer Buffer immediately prior to use.
- **Gel**—Use the gel immediately following the run. **Do not soak the gel in transfer buffer.**

Continued on next page

Western Transfer Using the XCell II™ Blot Module

Wear gloves while performing the blotting procedure to prevent contamination of gels and membranes, and exposure to irritants commonly used in electrotransfer.

Transferring One Gel

1. After opening the gel cassette as described on page 32, remove wells with the Gel Knife.
2. Place a piece of pre-soaked filter paper on top of the gel, and lay just above the slot in the bottom of the cassette, leaving the "foot" of the gel uncovered. Keep the filter paper saturated with the transfer buffer and remove all trapped air bubbles by gently rolling over the surface using a glass pipette as a roller.
3. Turn the plate over so the gel and filter paper are facing downwards over a gloved hand or clean flat surface.
4. Use the Gel Knife to push the foot out of the slot in the plate and the gel will fall off.
5. When the gel is on a flat surface, cut the "foot" off the gel with the gel knife.
6. Wet the surface of the gel with transfer buffer and position the pre-soaked transfer membrane on the gel, ensuring all air bubbles have been removed.
7. Place another pre-soaked anode filter paper on top of the membrane. Remove any trapped air bubbles.
8. Place two soaked blotting pads into the cathode (-) core of the blot module. The cathode core is the deeper of the two cores and the corresponding electrode plate is a darker shade of gray. Carefully pick up the gel membrane assembly and place on pad in the same sequence, such that the gel is closest to the cathode plate.
9. Add enough pre-soaked blotting pads to rise to 0.5 cm over rim of cathode core. Place the anode (+) core on top of the pads. The gel/membrane assembly should be held securely between the two halves of the blot module ensuring complete contact of all components.
10. Position the gel membrane sandwich and blotting pads in the cathode core of the XCell II™ Blot Module to fit horizontally across the bottom of the unit. There should be a gap of approximately 1 cm at the top of the electrodes when the pads and assembly are in place.
11. Hold the blot module together firmly and slide it into the guide rails on the lower buffer chamber. The blot module will only fit into the unit one way, so the (+) sign can be seen in the upper left hand corner of the blot module. Properly placed, the inverted gold post on the right hand side of the blot module will fit into the hole next to the upright gold post on the right side of the lower buffer chamber.
12. Place the gel tension wedge so that its vertical face is against the blot module. Lock the gel tension wedge by pulling the lever forward.

Continued on next page

Western Transfer Using the XCell II™ Blot Module, continued

13. Fill the blot module with 1X Transfer Buffer until the gel/membrane sandwich is covered in this buffer. Do not fill all the way to the top as this will only generate extra conductivity and heat.
14. Fill the outer buffer chamber with deionized water by pouring approximately 650 ml in the gap between the front of the blot module and the front of the lower buffer chamber. The water level should reach approximately 2 cm from the top of the lower buffer chamber. This serves to dissipate heat produced during the run.
15. Place the lid on top of the unit.
16. With the power turned off, plug the red and black leads into the power supply. Refer to **Recommended Transfer Conditions** on the next page for transfer conditions.

Transferring Two Gels in One Blot Module

1. Repeat Steps 1–6 above twice to make two gel-membrane assemblies.
2. Place two pre-soaked pads on cathode shell of blot module. Place first gel-membrane assembly on pads in correct orientation, so gel is nearest cathode plate. (See Diagram 2).
3. Add another pre-soaked blotting pad on top of first membrane assembly.
4. Position second gel-membrane assembly on top of blotting pad in the correct orientation so that the gel is nearest the cathode side.
5. Proceed with steps 8–13 from **Transferring One Gel**.
6. Refer to **Recommended Transfer Conditions** on the next page for transfer conditions.

Figure 1

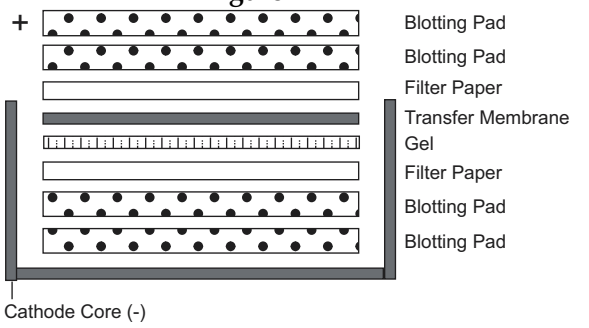
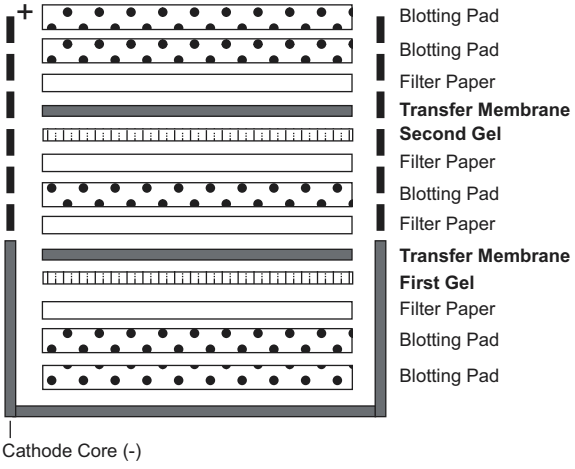


Figure 2



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Blotting Novex® Pre-Cast Gels, Continued

Recommended Transfer Conditions

The transfer conditions for Novex® Pre-Cast Gels using the XCell II™ Blot Module are listed in the table below.

Note: The expected current listed in the table is for transferring one gel. If you are transferring two gels in the blot module, the expected current will double.

Gel	Transfer Buffer	Membrane	Power Conditions
Tris-Glycine Gel Tricine Gel	1X Tris-Glycine Transfer Buffer with 20% methanol	Nitrocellulose or PVDF	25 V constant for 1-2 hours Expected Current Start: 100 mA
IEF Gel	1X Tris-Glycine Transfer Buffer with 20% methanol	Nitrocellulose or PVDF	25 V constant for 1 hour Expected Current Start: 65-85 mA
	0.7% Acetic acid pH 3.0 See next page for details on this alternate transfer protocol	Nitrocellulose or PVDF	10 V constant for 1 hour Expected Current Start: 65-85 mA
TBE Gel	0.5X TBE Running Buffer	Nylon	30 V constant for 1 hour Expected Current Start: 39 mA End: 35 mA
TBE-Urea Gel	0.5X TBE Running Buffer	Nylon	30 V constant for 1 hour Expected Current Start: 39 mA End: 35 mA
DNA Retardation Gel	0.5X TBE Running Buffer	Nylon	30 V constant for 1 hour Expected Current Start: 39 mA End: 35 mA

Continued on next page

Blotting IEF Gels

Novex® IEF Gels are composed of 5% polyacrylamide and are more susceptible to hydrolysis due to the heat generated with the recommended blotting protocol. The following protocol has been optimized to prevent hydrolysis and effective transfer of basic proteins due to the low pH of the transfer buffer.

1. Chill 0.7% acetic acid which will be used later for transfer.
2. After electrophoresis of the gel, equilibrate the gel in 0.7% acetic acid for 10 minutes.

   Tip: The 5% polyacrylamide gels are more sticky and difficult to handle than higher percentage polyacrylamide gels. To lift the gel from the equilibration solution, submerge the filter paper under the gel while the gel is floating in the equilibration solution. When the gel is in the correct position, lift up the filter paper to attach the gel to the filter paper. This prevents the gel from sticking to the filter paper before it is in the proper position and avoids handling of the gel.
3. Assemble the gel/membrane sandwich as described previously, except in a **reverse order** so that the membrane is on the cathode (-) side of the gel. This is the opposite of a typical western blotting protocol, where the negatively charged protein will migrate toward the anode (+) during the transfer.
4. Transfer for 1 hour at 10 V constant.

Blotting Native Gels

During SDS-PAGE all proteins have a net negative charge due to the SDS in the sample buffer and the running buffer. Proteins separated during native gel electrophoresis do not have a net charge which may cause problems during the transfer. Some native proteins may have a higher pI than the pH of the Tris-Glycine Transfer Buffer used in standard transfer protocols. Guidelines are provided below to increase the transfer efficiency of native proteins.

- Increasing the pH of the transfer buffer to 9.2 (25 mM Tris Base, 25 mM glycine, pH 9.2), allows proteins with pI below 9.2 to transfer towards the anode electrode
- Place a membrane on both sides of the gel if you are using the regular Tris-Glycine Transfer Buffer, pH 8.3. If there are any proteins that are more basic than the pH of the transfer buffer, they will be captured on the membrane placed on the cathode side of the gel
- Incubate the gel in 0.1% SDS for 15 minutes before blotting with Tris-Glycine Transfer Buffer. The small amount of SDS will render enough charge to the proteins so they can move unidirectionally towards the anode and in most cases will not denature the protein

Native proteins may diffuse out of the membrane into the solution during the blocking or antibody incubation steps, as the native proteins tend to be more soluble. To prevent diffusion of the proteins out of the membrane, we recommend fixing the proteins to the membrane by air drying the membrane or incubating the membrane in 5-10% acetic acid for 15 minutes followed by rinsing the membrane with deionized water and then air drying.

By performing any of these two fixing methods the proteins will be sufficiently unfolded to expose hydrophobic sites and bind more efficiently to the membrane.

Calibrating Protein Molecular Weight

Introduction

The molecular weight of a protein can be determined based upon its relative mobility by constructing a standard curve with protein standards of known molecular weights.

The protein mobility in SDS-PAGE gels is dependent on the

- Length of the protein in its fully denatured state,
- SDS-PAGE buffer systems
- Secondary structure of the protein

An identical molecular weight standard may have slightly different mobility resulting in different apparent molecular weight when run in different SDS-PAGE buffer systems.

If you are using the Novex® protein molecular weight standards, see the apparent molecular weights of these standards on the Novex® Pre-Cast Gels listed on the next page to determine an apparent molecular weight of your protein.

Protein Secondary Structure

When using SDS-PAGE for molecular weight determination, slight deviations from the calculated molecular weight of a protein (calculated from the known amino acid sequence) can occur due to the retention of varying degrees of secondary structure in the protein, even in the presence of SDS. This phenomenon is observed in highly organized secondary structures (such as collagens, histones, or highly hydrophobic membrane proteins) and in peptides, where the effect of local secondary structure and amino acid side chains becomes magnified relative to the total size of the peptide.

Buffer Systems

Slight differences in protein mobilities also occur when the same proteins are run in different SDS-PAGE buffer systems. Each SDS-PAGE buffer system has a different pH, which affects the charge of a protein and its binding capacity for SDS. The degree of change in protein mobility is usually small in natural proteins but more pronounced with “atypical” or chemically modified proteins such as pre-stained standards.

Continued on next page

Calibrating Protein Molecular Weight, Continued

Assigned Apparent Molecular Weights

The apparent molecular weight values currently provided with the Novex® molecular weight standards were derived from the construction of a calibration curve in the Tris-Glycine SDS-PAGE System. We have now calculated and assigned apparent molecular weights for the Novex® protein standards in several buffer systems. Remember to use the one that matches your gel for the most accurate calibration of your protein.

The following charts summarize the approximate molecular weight values for the Novex® protein molecular weight standards when run in different buffer systems. You may generate calibration curves in your lab with any other manufacturer's standards.

MultiMark® Multi-Colored Standard	Tris-Glycine Gels (4-20%)	Tricine Gels (10-20%)
Myosin	250 kDa	208 kDa
Phosphorylase B	148 kDa	105 kDa
Glutamic Dehydrogenase	60 kDa	53 kDa
Carbonic Anhydrase	42 kDa	34 kDa
Myoglobin (Blue)	30 kDa	23 kDa
Myoglobin (Red)	22 kDa	17 kDa
Lysozyme	17 kDa	13 kDa
Aprotinin	6 kDa	7 kDa
Insulin	4 kDa	4 kDa

Mark 12™ Unstained Standard	Tris-Glycine Gels (4-20%)	Tricine Gels (10-20%)
Myosin	200 kDa	200 kDa
β-Galactosidase	116.3 kDa	116.3 kDa
Phosphorylase B	97.4 kDa	97.4 kDa
Bovine Serum Albumin	66.3 kDa	66.3 kDa
Glutamic Dehydrogenase	55.4 kDa	55.4 kDa
Lactate Dehydrogenase	36.5 kDa	36.5 kDa
Carbonic Anhydrase	31 kDa	31 kDa
Trypsin Inhibitor	21.5 kDa	21.5 kDa
Lysozyme	14.4 kDa	14.4 kDa
Aprotinin	6 kDa	6 kDa
Insulin B Chain	Unresolved Insulin	3.5 kDa
Insulin A Chain		2.5 kDa

Continued on next page

Calibrating Protein Molecular Weight, Continued

Assigned Apparent Molecular Weights, continued

SeeBlue® Pre-Stained Standard	Tris-Glycine Gel (4-20%)	Tricine Gel (10-20%)
Myosin	250 kDa	210 kDa
BSA	98 kDa	78 kDa
Glutamic Dehydrogenase	64 kDa	55 kDa
Alcohol Dehydrogenase	50 kDa	45 kDa
Carbonic Anhydrase	36 kDa	34 kDa
Myoglobin	30 kDa	23 kDa
Lysozyme	16 kDa	16 kDa
Aprotinin	6 kDa	7 kDa
Insulin	4 kDa	4 kDa

SeeBlue® Plus2 Pre-Stained Standard	Tris-Glycine Gel (4-20%)	Tricine Gel (10-20%)
Myosin	250 kDa	210 kDa
Phosphorylase B	148 kDa	105 kDa
BSA	98 kDa	78 kDa
Glutamic Dehydrogenase	64 kDa	55 kDa
Alcohol Dehydrogenase	50 kDa	45 kDa
Carbonic Anhydrase	36 kDa	34 kDa
Myoglobin	22 kDa	17 kDa
Lysozyme	16 kDa	16 kDa
Aprotinin	6 kDa	7 kDa
Insulin	4 kDa	4 kDa

Troubleshooting

Introduction

Review the information below to troubleshoot your experiments with Novex® Gels.

Problem	Cause	Solution
Run taking longer time	Running buffer too dilute	Make fresh running buffer as described in this manual and avoid adjusting the pH of the 1X running buffer.
Low or no current during the run	Incomplete circuit	• Remove the tape from the bottom of the cassette prior to electrophoresis. • Make sure the buffer covers the sample wells. • Check the wire connections on the buffer core to make sure the connections are intact.
Faint shadow or "ghost" band below the expected protein band	Ghost bands are caused due to a slight lifting of the gel from the cassette resulting in trickling of some sample beyond its normal migration point. Gel lifting off the cassette is caused due to: • Expired gels • Improper storage of gels	• Avoid using expired gels. Use fresh gels • Store the gels at the appropriate temperature (see page v).
Streaking of proteins	• Sample overload • High salt concentration in the sample • Sample precipitates • Contaminants such as membranes or DNA complexes in the sample	• Load the appropriate amount of protein as described on page 6. • Decrease the salt concentration of your sample using dialysis or gel filtration • Increase the concentration of SDS in your sample if necessary, to maintain the solubility of the protein. • Centrifuge or clarify your sample to remove particulate contaminants
Bands in the outer lane of the gel are curving upwards	• Concentrated buffer used • Expired gels used • High voltage used	• The pre-made buffers are supplied as concentrate. Dilute the buffers as described in this manual. • Avoid using gels after the expiration date. • Electrophorese the gel using conditions described on page 30.

Continued on next page

Problem	Cause	Solution
Bands in the outside lanes of the gel "smiling"	Expired gels used causing the acrylamide to break down in the gel	Avoid using gels after the expiration date. Use fresh gels.
Bands are running as U shape rather than a flat band	Samples are loaded on the gel and not electrophoresed immediately resulting in sample diffusion	Load samples on to the gel immediately before electrophoresis.
Bands appear to be "funneling" or getting narrower as they progress down the gel	Proteins are over-reduced causing the proteins to be negatively charged and repel each other.	Reduce the proteins using DTT or β-mercaptoethanol as described on page 8.
Dumbbell shaped bands after electrophoresis	Loading a large volume of sample causing incomplete stacking of the entire sample. This effect is intensified for larger proteins	Load the appropriate volume of sample per well as described on page 6. If your sample is too dilute, concentrate the sample using salt precipitation or ultrafiltration.
For TBE-Urea gels High background and smeared bands or abnormal band shapes	• RNase contamination	• Always wear gloves and use sterile techniques to prevent RNase contamination.
	• Sample renatured	• Heat the sample for 3 minutes at 70°C and keep the sample in ice to prevent renaturation. Proceed to electrophoresis immediately after loading.
	• Sample overloaded	• Recommended DNA load is 0.16-0.33 µg/band.
	• Urea not completely flushed from the wells	• Be sure to thoroughly flush urea out of the wells prior to loading the sample.

Appendix

Accessory Products

Electrophoresis Reagents

Ordering information on a variety of electrophoresis reagents and apparatus available from Invitrogen is provided below. For more information, visit our Web site at www.invitrogen.com or call Technical Service (see page 66).

Product	Quantity	Catalog no.
XCell *SureLock*™ Mini-Cell	1 unit	EI0001
XCell II™ Blot Module	1 unit	EI9051
PowerEase® 500 Power Supply	1 unit	EI8600
DryEase® Mini-Gel Drying System	1 Kit	NI2387
StainEase® Staining Tray	2/pack	NI2400
Gel-Dry™ Drying Solution	500 ml	LC4025
NuPAGE® Transfer Buffer (20X)	125 ml	NP0006
Novex®Tris-Glycine SDS Running Buffer (10X)	500 ml	LC2675
NuPAGE® Sample Reducing Agent (10X)	250 µl	NP0004
NuPAGE® LDS Sample Buffer (4X)	250 ml	NP0008
Novex® Tris-Glycine Transfer Buffer (25X)	500 ml	LC3675
Novex® Tris-Glycine Native Running Buffer (10X)	500 ml	LC2672
Novex® Tris-Glycine Native Sample Buffer (2X)	20 ml	LC2673
Novex® Tris-Glycine SDS Sample Buffer (2X)	20 ml	LC2676
Novex® Tricine SDS Running Buffer (10X)	500 ml	LC1675
Novex® Tricine SDS Sample Buffer (2X)	20 ml	LC1676
Novex® Zymogram Renaturing Buffer (10X)	500 ml	LC2670
Novex® Zymogram Developing Buffer (10X)	500 ml	LC2671
Novex® TBE Running Buffer (5X)	1 L	LC6675
Novex® Hi-Density TBE Sample Buffer (5X)	10 ml	LC6678
Novex® TBE-Urea Sample Buffer (2X)	10 ml	LC6876
Novex® Prep TBE-Urea Sample Buffer (2X)	20 ml	LC6877
NuPAGE® Novex 4-12% Bis-Tris ZOOM® Gel	1 gel	NP0330BOX
Novex® 4-20% Tris-Glycine ZOOM® Gel	1 gel	EC60261BOX
UltraPure™ Agarose	100 g	15510-019
Ntirocellulose (0.45µm)	20 membrane/filter papers	LC2000
Invitrolon™ PVDF (0.45 µm)	20 membrane/filter papers	LC2005
Nylon (0.45 µm)	20 membrane/filter papers	LC2003

Continued on next page

Protein Stains and Standards

Ordering information for stains and protein molecular weight standards is provided below. For more information, visit our Web site at www.invitrogen.com or contact Technical Service (see page 66).

Product	Application	Quantity	Catalog no.
SimplyBlue™ Safe-Stain	Fast, sensitive, safe Coomassie® G-250 staining of proteins in polyacrylamide gels	1 L	LC6060
SilverQuest™ Silver Staining Kit	Sensitive silver staining of proteins compatible with mass spectrometry analysis	1 Kit	LC6070
Colloidal Blue Staining Kit	Sensitive colloidal Coomassie® G-250 staining of proteins in polyacrylamide gels	1 Kit	LC6025
SilverXpress® Silver Staining Kit	High-sensitivity, low background protein and nucleic acid silver staining	1 Kit	LC6100
Mark 12™ Unstained Standard	For estimating the apparent molecular weight of proteins	1 ml	LC5677
MagicMark™ Western Standard	For protein molecular weight estimation on western blots	250 µl	LC5600
SeeBlue® Pre-Stained Standard	For monitoring the progress of your run and evaluating transfer efficiency	500 µl	LC5625
SeeBlue® Plus2 Pre-Stained Standard	For visualizing protein molecular weight range and evaluating transfer efficiency	500 µl	LC5925
MultiMark® Multi-Colored Standard	For visualizing protein molecular weight range and evaluating transfer efficiency	500 µl	LC5725
BenchMark® Protein Ladder	For estimating the apparent molecular weight of proteins	2 x 250 µl	10747-012
IEF Marker 3-10	For determining the pI of proteins	500 µl	39212-01

Nucleic Acid Markers

A large variety of Nucleic Acid Markers are available from Invitrogen. Ready-Load™ format (pre-mixed with loading buffer) nucleic acid markers are also available for your convenience. For more information, visit our Web site at www.invitrogen.com or contact Technical Service (see page 66).

Recipes

Tris-Glycine SDS Running Buffer

The Tris-Glycine SDS Running Buffer is available from Invitrogen (see page 57)

25 mM Tris Base
192 mM Glycine
0.1% SDS
pH 8.3

1. To prepare 1000 ml of 10 X Tris-Glycine SDS Running Buffer, dissolve the following reagents to 900 ml ultrapure water:

Tris Base	29 g
Glycine	144 g
SDS	10 g

2. Mix well and adjust the volume to 1000 ml with ultrapure water.
3. Store at room temperature. The buffer is stable for 6 months when stored at room temperature.
4. For electrophoresis, dilute this buffer to 1X with water (see page 11). The pH of the 1X solution is 8.3. Do not use acid or base to adjust the pH.

Tris-Glycine Native Running Buffer

The Tris-Glycine Native Running Buffer is available from Invitrogen (see page 57)

25 mM Tris base
192 mM Glycine
pH 8.3

1. To prepare 1000 ml of 10 X Tris-Glycine Native Running Buffer, dissolve the following reagents to 900 ml ultrapure water:

Tris Base	29 g
Glycine	144 g

2. Mix well and adjust the volume to 1000 ml with ultrapure water.
3. Store at room temperature. The buffer is stable for 6 months when stored at room temperature.
4. For native electrophoresis, dilute this buffer to 1X with water (see page 11). The pH of the 1X solution is 8.3. Do not use acid or base to adjust the pH.

Continued on next page

Tris-Glycine SDS Sample Buffer

The Tris-Glycine SDS Sample Buffer is available from Invitrogen (see page 57)

63 mM Tris HCl
10% Glycerol
2% SDS
0.0025% Bromophenol Blue
pH 6.8

1. To prepare 10 ml of 2X Tris-Glycine SDS Sample Buffer, mix the following reagents :

0.5 M Tris-HCl, pH 6.8	2.5 ml
Glycerol	2 ml
10% (w/v) SDS	4 ml
0.1% (w/v) Bromophenol Blue	0.5 ml

2. Adjust the volume to 10 ml with ultrapure water.
3. Store at +4°C. The buffer is stable for 6 months when stored at +4°C.

Tris-Glycine Native Sample Buffer

The Tris-Glycine Native Sample Buffer is available from Invitrogen (see page 57)

100 mM Tris HCl
10% Glycerol
0.0025% Bromophenol Blue
pH 8.6

1. To prepare 10 ml of 2X Tris-Glycine Native Sample Buffer, mix the following reagents :

0.5 M Tris HCl, pH 8.6	4 ml
Glycerol	2 ml
0.1% (w/v) Bromophenol Blue	0.5 ml

2. Adjust the volume to 10 ml with ultrapure water.
3. Store at +4°C. The buffer is stable for 6 months when stored at +4°C.

Tris-Glycine Transfer Buffer

The Tris-Glycine Transfer Buffer is available from Invitrogen (see page 57)

12 mM Tris Base
96 mM Glycine
pH 8.3

1. To prepare 500 ml of 25 X Tris-Glycine Transfer Buffer, dissolve the following reagents in 400 ml ultrapure water:

Tris Base	18.2 g
Glycine	90 g

2. Mix well and adjust the volume to 500 ml with ultrapure water.
3. Store at room temperature. The buffer is stable for 6 months when stored at room temperature.
4. For blotting, dilute this buffer as described on page 46. The pH of the 1X solution is 8.3. Do not use acid or base to adjust the pH.

Continued on next page

Tricine SDS Sample Buffer

The Tricine SDS Sample Buffer is available from Invitrogen (see page 57)

450 mM Tris HCl
12% Glycerol
4% SDS
0.0025% Coomassie® Blue G
0.0025% Phenol Red
pH 8.45

1. To prepare 10 ml of 2X Tricine SDS Sample Buffer, mix the following reagents:

3 M Tris HCl, pH 8.45	3 ml
Glycerol	2.4 ml
SDS	0.8 g
0.1% Coomassie® Blue G	0.5 ml
0.1% Phenol Red	0.5 ml

2. Mix well and adjust the volume to 10 ml with ultrapure water.
3. Store at +4°C. The buffer is stable for 6 months when stored at +4°C.

Tricine SDS Running Buffer

The Tricine SDS Running Buffer is available from Invitrogen (see page 57)

100 mM Tris base
100 mM Tricine
0.1% SDS
pH 8.3

1. To prepare 1000 ml of 10 X Tricine SDS Running Buffer, dissolve the following reagents in 900 ml deionized water:

Tris Base	121 g
Tricine	179 g
SDS	10 g

2. Mix well and adjust the volume to 1000 ml with ultrapure water.
3. Store at room temperature. The buffer is stable for 6 months when stored at room temperature.
4. For electrophoresis, dilute this buffer to 1X with water (see page 13). The pH of the 1X solution is 8.3. Do not use acid or base to adjust the pH.

Zymogram Renaturing Buffer

The Zymogram Renaturing Buffer is available from Invitrogen (see page 57)

2.7% (w/v) Triton X-100

1. To prepare 500 ml of 10X Zymogram Renaturing Buffer, add 135 g Triton X-100 to 900 ml ultra pure water.
2. Mix well and adjust the volume to 500 ml with ultrapure water.
3. Store at room temperature. The buffer is stable for 6 months when stored at room temperature.

Continued on next page

Zymogram Developing Buffer

The Zymogram Developing Buffer is available from Invitrogen (see page 57)

50 mM Tris base
40 mM HCl
200 mM NaCl
5 mM $CaCl_2$
0.02% (w/v) Brij 35

1. To prepare 500 ml of 10X Zymogram Developing Buffer, dissolve the following reagents in 400 ml deionized water:

Tris Base	30.2 g
6N HCl	33 ml
NaCl	58.5 g
$CaCl_2.2H_2O$	3.7 g
Brij 35	10 g

2. Mix well and adjust the volume to 500 ml with ultrapure water.
3. Store at room temperature. The buffer is stable for 6 months when stored at room temperature.
4. For developing the zymogram gel, dilute this buffer to 1X with water (see page 17).

IEF Sample Buffer pH 3-7

The IEF Sample Buffer pH 3-7 is available from Invitrogen (see page 57)

40 mM Lysine (free base)
15% Glycerol

1. To prepare 10 ml of 2X IEF Sample Buffer pH 3-7, mix the following reagents:

10X IEF Cathode Buffer, pH 3-7 (see next page)	2 ml
Glycerol	3 ml

2. Mix well and adjust the volume to 10 ml with ultrapure water.
3. Store at +4°C. The buffer is stable for 6 months when stored at +4°C.

IEF Sample Buffer, pH 3-10

The IEF Sample Buffer pH 3-10 is available from Invitrogen (see page 57)

20 mM Lysine (free base)
20 mM Arginine (free base)
15% Glycerol

1. To prepare 10 ml of 2X IEF Sample Buffer pH 3-7, mix the following reagents:

10X IEF Cathode Buffer, pH 3-10 (see next page)	2 ml
Glycerol	3 ml

2. Mix well and adjust the volume to 10 ml with ultrapure water.
3. Store at +4°C. The buffer is stable for 6 months when stored at +4°C.

Continued on next page

IEF Cathode Buffer, pH 3-7

The IEF Cathode Buffer pH 3-7 is available from Invitrogen (see page 57)

40 mM Lysine (free base)

1. To prepare 100 ml of 10X IEF Cathode Buffer pH 3-7, dissolve 5.8 g of Lysine (free base) in 100 ml of ultrapure water.
2. Store at +4°C. The buffer is stable for 6 months when stored at +4°C.

IEF Cathode Buffer, pH 3-10

The IEF Cathode Buffer pH 3-10 is available from Invitrogen (see page 57)

20 mM Lysine (free base)
20 mM Arginine (free base) You can use D, L, or D/L form of arginine
pH 10.1

1. To prepare 100 ml of 10X IEF Cathode Buffer pH 3-10, dissolve 2.9 g of Lysine (free base) and 3.5 g of Arginine (free base) in 100 ml of ultrapure water.
2. Store at +4°C. The buffer is stable for 6 months when stored at +4°C.

IEF Anode Buffer

The IEF Anode Buffer is available from Invitrogen (see page 57)

7 mM Phosphoric acid

1. To prepare 100 ml of 50X IEF Anode Buffer, mix 2.4 ml of 85% phosphoric acid with 97.6 ml of ultrapure water.
2. Store at room temperature. The buffer is stable for 6 months when stored at room temperature.

TBE Running Buffer

The TBE Running Buffer is available from Invitrogen (see page 57)

89 mM Tris base
89 mM Boric acid
2 mM EDTA (free acid)
pH 8.3

1. To prepare 1000 ml of 5X TBE Running Buffer, dissolve the following reagents in 900 ml deionized water:

Tris Base	54 g
Boric acid	27.5 g
EDTA (free acid)	2.9 g

2. Mix well and adjust the volume to 1000 ml with ultrapure water.
3. Store at room temperature. The buffer is stable for 6 months when stored at room temperature.
4. For electrophoresis, dilute this buffer to 1X with water as described on page 25. The pH of the 1X solution is 8.3. Do not use acid or base to adjust the pH.

Continued on next page

Hi-Density TBE Sample Buffer

The Hi-Density TBE Sample Buffer is available from Invitrogen (see page 57)

18 mM Tris base
18 mM Boric acid
0.4 mM EDTA (free acid)
3% Ficoll™ Type 400
0.02% Bromophenol Blue
0.02% Xylene Cyanol

1. To prepare 10 ml of 5X Hi-Density TBE Sample Buffer, dissolve the following reagents in 9 ml deionized water:

5X TBE Running Buffer (see previous page)	2 ml
Ficoll™ Type 400	1.5 g
1% Bromophenol Blue	1 ml
1% Xylene Cyanol	1 ml

2. Mix well and adjust the volume to 10 ml with ultrapure water.
3. Store at +4°C. The buffer is stable for 6 months when stored at+4°C.

TBE-Urea Sample Buffer

The TBE-Urea Sample Buffer is available from Invitrogen (see page 57)

45 mM Tris base
45 mM Boric acid
1 mM EDTA (free acid)
6% Ficoll™ Type 400
3.5 M Urea
0.005% Bromophenol Blue
0.025% Xylene Cyanol

1. To prepare 10 ml of 2X TBE-Urea Sample Buffer, dissolve the following reagents in 9 ml deionized water:

5X TBE Running Buffer (see previous page)	2 ml
Ficoll™ Type 400	1.2 g
1% Bromophenol Blue	1 ml
1% Xylene Cyanol	0.5 ml
Urea	4.2 g

2. Mix well and adjust the volume to 10 ml with ultrapure water.
3. Store at +4°C. The buffer is stable for 3 months when stored at+4°C.

Continued on next page

Prep TBE–Urea Sample Buffer

The Prep TBE–Urea Sample Buffer is available from Invitrogen (see page 57)

45 mM Tris base
45 mM Boric acid
1 mM EDTA (free acid)
6% Ficoll™ Type 400
3.5 M Urea

1. To prepare 10 ml of 2X Prep TBE–Urea Sample Buffer, dissolve the following reagents in 9 ml deionized water:

5X TBE Running Buffer (see page 63)	2 ml
Ficoll™ Type 400	1.2 g
Urea	4.2 g

2. Mix well and adjust the volume to 10 ml with ultrapure water.
3. Store at +4°C. The buffer is stable for 6 months when stored at+4°C.

Technical Service

World Wide Web

Visit the Invitrogen Web Resource using your World Wide Web browser. At the site, you can:

- Get the scoop on our hot new products and special product offers
- View and download vector maps and sequences
- Download manuals in Adobe® Acrobat® (PDF) format
- Explore our catalog with full color graphics
- Obtain citations for Invitrogen products
- Request catalog and product literature

Once connected to the Internet, launch your Web browser (Internet Explorer 5.0 or newer or Netscape 4.0 or newer), then enter the following location (or URL):

http://www.invitrogen.com

...and the program will connect directly. Click on underlined text or outlined graphics to explore. Don't forget to put a bookmark at our site for easy reference!

Contact Us

For more information or technical assistance, call, write, fax, or email. Additional international offices are listed on our Web page (www.invitrogen.com).

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MSDS Requests

To request an MSDS, visit our Web site (www.invitrogen.com) and follow the instructions below.

1. On the home page, go to 'Technical Resources' and select 'MSDS Requests'.
2. Follow instructions on the page and fill out all the required fields.
3. To request additional MSDSs, click the 'Add Another' button.
4. All requests will be faxed unless another method is selected.
5. When you are finished entering information, click the 'Submit' button. Your MSDS will be sent within 24 hours.

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