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Novex® Pre-Cast Gel Electrophoresis Guide

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

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User Manual

Contents

General Information

Purpose of the Guide

A variety of pre-cast gels for use with the XCell *SureLock*™ Mini-Cell 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).

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 Support (see page 76) or download the manuals from our website 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 significantly 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 Invitrogen website for the SDS (see page 76).

Intended Use

For research use only. Not intended for human or animal diagnostic or therapeutic uses.

Overview of Electrophoresis

Introduction

Electrophoresis is a simple, rapid, and sensitive analytical tool for separating proteins and nucleic acids based on their physical characteristics (mass, isoelectric point, etc.).

Most biological molecules carry a net charge at any pH other than their isoelectric point and migrate at a rate proportional to their charge density in an electrical field.

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

- Field strength
- Net charge on the molecule
- Size and shape of the molecule
- Ionic strength
- Properties of the medium through which the molecules migrate (e.g., viscosity, pore size)

Support Matrix

Polyacrylamide and agarose are two types of support matrices used in electrophoresis. The support matrix is a porous media that acts as a molecular sieve. The sieving function depends on the pore size, and concentration of the matrix. Agarose has a large pore size and is ideal for separating macro-molecules such as nucleic acids and protein complexes. Polyacrylamide has a smaller pore size and is ideal for separating proteins and smaller nucleic acids.

Polyacrylamide Gel Electrophoresis (PAGE)

Polyacrylamide gels are formed by the polymerization of acrylamide monomers into long chains, crosslinked by bifunctional compounds such as N,N-methylene-bisacrylamide (bis) that react with the free functional groups at the chain termini.

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

%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, allowing 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.

Continuous buffer systems utilize a single buffer for the gel and the running buffer.

Discontinuous buffer systems (Ornstein 1964) utilize different gel buffers and running buffer. In addition, two gel layers of different pore size, the stacking and separating gel, are used. Electrophoresis using a discontinuous buffer system allows concentration of the sample to a narrow region prior to separation, resulting in sharper bands and higher resolution.

Electrophoresis Sample Conditions

Depending upon the application, electrophoresis can be performed under the following conditions:

Denaturing

Electrophoresis is performed under denaturing conditions using an anionic detergent such as sodium dodecylsulfate (SDS). SDS denatures and unfolds the proteins by binding the hydrophobic portions of the protein 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)

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

Reducing

Electrophoresis is performed under reducing conditions using reducing agents such as dithiothreitol (DTT) or β-mercaptoethanol (β-ME). The reducing agents cleave any disulfide bonds between cysteine residues resulting in complete separation of denatured proteins into their individual subunits.

Continued on next page

Power Supply Considerations for Electrophoresis

In electrical terms, the process of electrophoresis is closely associated with the following equations derived from Ohm's Law:

Voltage = Current × Resistance (V=IR)
Wattage = Current × Voltage (W=IV)

Resistance

The electrical resistance of the assembled electrophoresis cell is dependent on buffer conductivity, gel thickness, temperature, 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 discontinuous buffer systems (and to a lesser extent in continuous buffer systems) resistance increases over the course of electrophoresis. This occurs in the Tris-Glycine buffer system as highly conductive chloride ions in the gel are replaced by less conductive glycine ions from the running buffer.
- Resistance decreases as the temperature increases.

Voltage

The velocity of an ion in an electric field varies in proportion to the field strength (Volts per unit distance). The higher the voltage, the faster an ion moves. For most applications, **we recommend a constant voltage setting**.

- A constant voltage setting allows the current and power to decrease over the course of electrophoresis, providing a safety margin in case of a break in the system.
- The constant voltage setting does not need adjustment to account for differences in number or thickness of gels being electrophoresed.

Current

For a given gel/buffer system, at a given temperature, current varies in proportion to the field strength (voltage) and/or cross-sectional area (thickness and/or number of gels). When using a constant current setting, migration starts slow, and accelerates over time, thus favoring stacking in discontinuous gels.

When running under constant current, set a voltage limit on the power supply at, or slightly above the maximum expected voltage to avoid unsafe conditions. At constant current voltage increases as resistance increases. If a local fault condition occurs (e.g., a bad connection), high local resistance may cause the voltage to reach the maximum for the power supply, leading to overheating and damage of the electrophoresis cell.

Power

Wattage measures the rate of energy conversion, which is manifest as heat generated by the system. Using constant power ensures that the total amount of heat generated by the system remains constant throughout the run, but results in variable mobility since voltage increases and current decreases over the course of the run. Constant power is typically used when using IEF strips.

When using constant power, set the voltage limit slightly above the maximum expected for the run. High local resistance can cause a large amount of heat to be generated over a small distance, damaging the electrophoresis cell and gels.

Novex® Gel Specifications

Introduction

The Novex® Pre-Cast Gel cassette is 10 cm × 10 cm in size, and designed for use with the XCell *SureLock*™ Mini-Cell and XCell6™ MultiGel Unit (see page 63 for ordering information).

Novex® Pre-Cast Gels are available for resolving proteins in the range of 2–500 kDa and nucleic acids in the range of 10–3,000 bp, depending upon the type and acrylamide percentage of the gel. Refer to **Gel Selection** (page 6) for details on applications and migration patterns.

Specifications

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

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Novex® Gel Specifications, Continued

Novex® Gel Formulations

All Novex® Pre-Cast gels are made with high purity reagents. The gels for DNA analysis are DNase-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 Charts** on (page 72).

Gel Selection

Choosing a Gel for Your Application

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

Review the following section, and **Well Volume** (page 8) to determine the type of gel that is best suited for your application.

Refer to the Novex® Gel Migration Charts (see page 72) to find the gel with the region of maximum resolution best suited for your sample. The leading protein molecules should migrate about 70% of the length of gel for best resolution.

Protein Separation Applications

Separation of proteins over a wide range of molecular weights

Use Novex® Tris-Glycine Gels for separating proteins over a wide molecular weight range (6–200 kDa) under denaturing or non-denaturing conditions.

Resolve large molecules with low percentage gels, and small molecules with high percentage gels. If the molecular weight of the molecule is unknown, or the sample contains a wide range of molecules, use a gradient gel.

Separation of low molecular weight proteins and peptides

The Novex® Tricine Gels provide high resolution of low molecular weight proteins and peptides (2–200 kDa). Tricine gels give the best results under denaturing conditions.

Isoelectric focusing (IEF)

Use Novex® IEF Gels for native (vertical) IEF of proteins. The pH 3–10 gels have a pI performance range of 3.5–8.5 and pH 3–7 gels have a pI performance range of 3.0–7.0.

Protease detection

The Novex® Zymogram Gels are used for detecting and characterizing proteases that utilize casein or gelatin as the substrate. Proteins are run under denaturing conditions and then renatured for enzymatic activity.

2D separation of proteins

The ZOOM® Gels are specifically designed for second dimension electrophoresis of 7.0 cm IPG strips. Gels with 2D wells can also be used, but only accommodate IPG strips of 6.5 cm.

Continued on next page

Gel Selection, Continued

Nucleic Acid Separation Applications

Nucleic acid analysis

The Novex® Pre-Cast Gels are capable of resolving nucleic acids in the range of 10–3000 bp.

Novex® TBE Gels are used to perform analysis of DNA fragments from restriction digest and PCR products, Southern analysis, and primer analysis.

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.

Gel shift assays

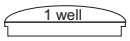
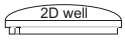
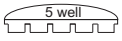
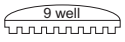
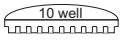
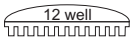
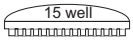
The Novex® 6% DNA Retardation Gels are used to perform gel shift assays.

Well Volume

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	

Choosing the Appropriate Well for Your Application

Choose the type of well for your application based upon the volume of your sample. The more wells a comb has, and the thinner the gel is, the lower the sample loading volume.

Note: Proteins transfer out of a 1.0 mm gel more easily than from a 1.5 mm gel.

Gel Staining

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 35–43) for silver staining of Novex® Gels.

Novex® Pre-Cast Gels are compatible with any of the standard Coomassie staining procedures. Protocols that are accelerated by heat are preferable, as heat can fix proteins (especially smaller peptides). The SimplyBlue™ SafeStain (see page 36) and Novex® Colloidal Blue Staining Kit (see page 37) are recommended for staining Novex® Gels.

Stain Type	Sensitivity	Gel Type Compatibility	Application
Coomassie Blue	100–500 ng	Tris-Glycine, Bis-Tris, Tricine, native	General
Coomassie Fluor™ Orange	8–16 ng		
Colloidal Coomassie Blue	<10 ng		
SimplyBlue™ SafeStain	5 ng		
SilverXpress®	1 ng	Tris-Glycine, Bis-Tris, Tricine, TBE	Low sample quantity, Nucleic acid
SilverQuest™	0.3–2.5 ng 0.3–0.9 ng (50 bp)	Bis-Tris, Tricine, TBE	
SYPRO® Ruby	0.25–1 ng	Tris-Glycine, Bis-Tris, Tricine, native	Low sample quantity, Nucleic acid, Mass Spec
Pro-Q® Diamond	1–16 ng	Tris-Glycine, Bis-Tris	Phosphoprotein
Pro-Q® Emerald	0.5–3 ng	Tris-Glycine	Glycoprotein
Ethidium Bromide	10 ng (50 bp)	TBE	Nucleic acid
SYBR® Green	60 pg (dsDNA) 100–300 pg (ssDNA) 1–2 ng (24 bp)	TBE	Nucleic acid

Methods

General Guidelines for Preparing Samples and Buffers

Introduction

The XCell *SureLock*™ Mini-Cell and a power supply are needed to perform electrophoresis with Novex® Pre-Cast gels. Additional reagents supplied by the user are described for each individual protocol.

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 are described in the sections for each individual type of gel.

Recommended Buffers

The recommended running buffer and sample buffer for each Novex® Pre-Cast Gel is listed in the table below. Prepare your sample in the appropriate sample buffer such that the final concentration of the sample buffer is 1X.

Running buffer must be diluted to 1X final concentration before use.

See page 63 for ordering information on pre-mixed buffers. See pages 65–71 for recipes if you are preparing your own buffers.

Gel Type	Running Buffer	Sample Buffer
Novex® Tris-Glycine Gels (SDS-PAGE)	Tris-Glycine SDS Running Buffer (10X)	Tris-Glycine SDS Sample Buffer (2X)
Novex® Tris-Glycine Gels (Native-PAGE)	Tris-Glycine Native Running Buffer (10X)	Tris-Glycine Native Sample Buffer (2X)
Novex® Tricine Gels	Tricine SDS Running Buffer (10X)	Tricine SDS Sample Buffer (2X)
Novex® 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

When preparing samples for reducing gel electrophoresis, any of the following reducing agents may be used:

- NuPAGE® Reducing Agent (see page 63 for ordering information)
- Dithiothreitol (DTT), 50 mM final concentration
- β-mercaptoethanol, 2.5% final concentration
- tris(2-carboxyethyl)phosphine (TCEP), 50 mM final concentration

Add the reducing agent to the sample up to an hour before loading the gel. Avoid storing reduced samples for long periods, even if they are frozen. Reoxidation of samples occur 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.

High Salt Concentration in Samples

High salt concentrations result in increased conductivity that affects protein migration, and can result in gel artifacts in adjacent lanes containing samples with normal salt concentrations. Perform dialysis or precipitate and resuspend samples in lower salt buffer prior to electrophoresis.

Guanidine-HCl in Samples

Samples solubilized in guanidine-HCl have high ionic strength, and produce increased conductivity similar to high salt concentrations. In addition, guanidine precipitates in the presence of SDS leading to various types of gel artifacts. If possible, change the solubilization agent by dialysis prior to electrophoresis.

Cell Lysates

Take the following considerations into account when performing electrophoresis of cell lysates:

- Genomic DNA in the cell lysate may cause the sample to become viscous and affect protein migration patterns and resolution. Shear genomic DNA to reduce viscosity before loading the sample.
- Cells lysates contain soluble and insoluble fractions. The size of each fraction depends upon the type of sample being analyzed. The nature of the insoluble fraction may result in altered protein migration patterns and resolution. Separate the two fractions by centrifugation and load them on separate lanes for electrophoresis.
- If RIPA buffer is used in cell lysis, subsequent blotting of proteins <40 kDa may be inhibited due to the presence of Triton® X-100 in the buffer.

Tris-Glycine Gels

Tris-Glycine Discontinuous Buffer System

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

The Tris-Glycine discontinuous buffer systems utilizes three ions:

- Chloride ($^-$) from the gel buffer serves as a leading ion due to its high affinity to the anode relative to other anions in the system. The gel buffer ions are $Tris^+$ and Cl^- (pH 8.65).
- Glycine ($^-$) is the primary anion in 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

The following reagents are needed to perform electrophoresis with Novex® Tris-Glycine Gels. Ordering information for pre-mixed buffers is on page 63. If you are preparing your own buffers, recipes are provided on pages 65–66.

- Protein sample
- Deionized water
- Protein molecular weight markers

For denaturing electrophoresis

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

For non-denaturing (native) electrophoresis

- Novex® Tris-Glycine Native Sample Buffer
- Novex® Tris-Glycine Native Running Buffer

Preparing Running Buffer

Use 1X Tris-Glycine SDS Running Buffer for electrophoresis of denatured samples, or 1X Native Running Buffer for electrophoresis of native samples.

1. Prepare 1,000 mL of Running Buffer as described below:

Reagent	Amount
10X Novex® Tris-Glycine SDS or 10X Native Running Buffer	100 mL
Deionized Water	900 mL
Total Volume	1,000 mL

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

Continued on next page

Tris-Glycine Gels, Continued

Preparing Samples for Denaturing Electrophoresis

To separate proteins by mass alone, denature samples using SDS Sample Buffer and heating.

1. Prepare each sample 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. Heat the sample at 85°C for 2 minutes. Load the samples onto the gel immediately.

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

Preparing Samples for Native Electrophoresis

To separate proteins by charge:mass ratio in their native conformation, use non-denaturing (native) electrophoresis.

1. Prepare each sample as described below:

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

2. Load the samples onto the gel immediately. **Do not** heat samples for native electrophoresis.

Electrophoresis Conditions

See page 32 for instructions on running Novex® Pre-Cast Gels using the XCell *SureLock*™ Mini-Cell. Run the gel at 125 V constant. See page 33 for additional details on electrophoresis conditions.

Staining the Gel

Any of the techniques described on pages 35–46 are suitable for staining Novex® Tris-Glycine Gels after electrophoresis.

Tricine Gels

Tricine Buffer System

The Tricine system is a modification of the Tris-Glycine discontinuous buffer system (see page 12) developed by Schaegger and von Jagow (Schaegger and von Jagow, 1987) specifically designed for resolving peptides and low molecular weight proteins.

In the Tris-Glycine system, proteins are stacked in the stacking gel between the 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. Smaller proteins mix with DS ions in the zone of stacked DS micelles, resulting in fuzzy bands and decreased resolution. The mixing also interferes with the fixing and staining of smaller proteins.

To avoid this problem, the Tricine system uses a low pH gel buffer and replaces the trailing glycine ion with a fast moving tricine ion in the running buffer. The smaller proteins that previously migrated with the stacked DS micelles in the Tris-Glycine system become well separated from DS ions in the Tricine system, resulting in more efficient stacking and destacking of low molecular weight proteins, sharper bands, and higher resolution

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 because of a lower pH

Materials Supplied by the User

The following reagents are needed to perform electrophoresis with Novex® Tricine Gels. Ordering information for pre-mixed buffers is on page 63. If you are preparing your own buffers, recipes are provided on page 67.

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

Continued on next page

Tricine Gels, Continued

Preparing Running Buffer

Use 1X Novex® Tricine SDS Running Buffer for electrophoresis of Tricine gels.

1. Prepare 1,000 mL of Running Buffer as described below:

Reagent	Amount
Novex® Tricine SDS Running Buffer (10X)	100 mL
Deionized Water	900 mL
Total Volume	1,000 mL

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

Note

Novex® Tricine Gel are not compatible with buffers for Tris-Glycine gels.

- Samples run in Tris-Glycine SDS Sample Buffer are poorly resolved.
- Samples run in Tris-Glycine SDS Running Buffer take longer to complete and result in poor resolution of smaller proteins.

Preparing Samples

Protein samples for Tricine Gels can be denatured, or denatured and reduced.

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

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 samples onto the gel immediately.

   Note: For reduced sample, add the reducing agent immediately prior to electrophoresis to obtain the best results. Leave an empty lane between samples with and without reducing agent to prevent diffusion of the reducing agent into non-reduced sample lanes.

Electrophoresis Conditions

See page 32 for instructions on running Novex® Pre-Cast Gels using the XCell *SureLock*™ Mini-Cell. Run the gel at 125 V constant. See page 33 for additional details on electrophoresis conditions.

Staining the Gel

Any of the techniques described on pages 35–46 are suitable for staining Novex® Tricine Gels after electrophoresis.

Zymogram Gels

Zymogram Technique

Zymogram analysis is used for detecting and characterizing metalloproteinases, collagenases, and other proteases that can utilize casein or gelatin as a substrate. Protease samples are denatured in SDS buffer **under non-reducing conditions** and **without heating**, and run on a Zymogram Gel using Tris-Glycine SDS Running Buffer. After electrophoresis, 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 divalent metal cations required for enzymatic activity), and then stained and destained. Regions of protease activity appear as clear bands against a dark blue background where the protease has digested the substrate.

Types of Zymogram Gels

Three different types of Zymogram Gels are available from Invitrogen. Details are listed on the table below.

Gel Type	Separating Gel	Substrate	Sensitivity
Novex® Zymogram Gelatin Gel	10% Tris-Glycine gel	with 0.1% gelatin	10^{-6} units of collagenase
Novex® Zymogram Casein Gel	12% Tris-Glycine gel	β-casein	7×10^{-4} units of trypsin
Novex® Zymogram Blue Casein Gel	4–16% Tris-Glycine gel	blue-stained β-casein	1.5×10^{-3} units of trypsin

Materials Supplied by the User

The following reagents are needed to perform electrophoresis with Novex® Zymogram Gels. Ordering information for pre-mixed buffers is on page 63. If you are preparing your own buffers, recipes are provided on pages 65–68.

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

Important

- Do not treat zymogram samples with reducing agents. Some proteases are multiunit complexes that require the full subunit assembly for activity.
- Load 2–3 times the recommended amount of unstained molecular weight marker required for a Tris-Glycine Gel. The marker needs to stain intensely to be visualized against the dark background of the Zymogram Gel.
- Leave an empty lane between protein molecular weight markers containing reducing agent and protease sample lanes to prevent diffusion of the reducing agent into the protease lane.

Continued on next page

Preparing Running Buffer

Use 1X Novex® Tris-Glycine SDS Running Buffer for electrophoresis of protease samples on Zymogram Gels.

1. Prepare 1,000 mL of Running Buffer as follows:

Reagent	Amount
Novex® Tris-Glycine SDS Running Buffer (10X)	100 mL
Deionized Water	900 mL
Total Volume	1,000 mL

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

Preparing Samples

Prepared samples without reducing agents so that multiunit proteases migrate as a single unit that can be renatured after electrophoresis.

1. Prepare each sample 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 samples onto the gel immediately. **Do not heat samples for Zymogram Gels.**

Electrophoresis Conditions

See page 32 for instructions on running Novex® Pre-Cast Gels using the XCell *SureLock*™ Mini-Cell. Run the gel at 125 V constant. See page 33 for additional details on electrophoresis conditions.

Detecting Protease Activity

After completing electrophoresis, renature the enzyme and develop the Zymogram Gels to detect protease activity.

Requirements for the volume of Zymogram Renaturing Buffer and Zymogram Developing Buffer may vary, depending upon the size of your developing tray.

Preparing Renaturing Buffer

Up to two mini-gels can be treated with every 100 mL of 1X Novex® Zymogram Renaturing Buffer.

1. Prepare 100 mL of Renaturing Buffer as described below:

Reagent	Amount
Novex® Zymogram Renaturing Buffer (10X)	10 mL
Deionized Water	90 mL
Total Volume	100 mL

2. Mix thoroughly before use.

Continued on next page

Zymogram Gels, Continued

Preparing Developing Buffer

Up to two mini-gels can be treated with every 100 mL of 1X Novex® Zymogram Developing Buffer:

1. Prepare 100 mL of Developing Buffer as described below:

Reagent	Amount
Novex® Zymogram Developing Buffer (10X)	10 mL
Deionized Water	90 mL
Total Volume	100 mL

2. Mix thoroughly before use.

   Note: Gels will be treated with Developing Buffer twice, so additional buffer may be required, depending upon the size of the developing tray.

Developing Zymogram Gels

1. Remove the gel from the cassette, or remove the top gel plate, and allow the gel to remain on the bottom gel plate for support.
2. Incubate the gel in 1X Novex® Zymogram Renaturing Buffer for 30 minutes at room temperature with gentle agitation.
3. Decant the Zymogram Renaturing Buffer and add 1X Novex® Zymogram Developing Buffer to the gel.
4. Equilibrate the gel for 30 minutes at room temperature with gentle agitation.
5. Decant the Developing Buffer and add fresh 1X Novex® Zymogram Developing Buffer to the gel.
6. Incubate the gel at 37°C for at least 4 hours, or overnight for maximum sensitivity. The 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 36–37.

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

IEF Gels

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 thus, does not migrate further in an electric field.

IEF Gels are used to determine the isoelectric point (pI) of a protein and to detect minor changes in the protein due to post-translational modifications such as phosphorylation and glycosylation.

In IEF, 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 proteins migrates through the pH gradient, individual proteins are immobilized in the pH gradient as they approach their pI.

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.

2D Electrophoresis

Proteins separated on IEF Gels are suitable for use in two-dimensional (2D) electrophoresis using Novex® Tris-Glycine or NuPAGE® Gels with a 2D-well or ZOOM® format to separate focused proteins by mass.

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 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 gel is stained after 2D electrophoresis to visualize the separated proteins, 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

During IEF, proteins migrate in an electric field until a stable pH gradient is formed and the proteins settle into their pI. A high finishing voltage is applied to focus the proteins into narrow zones. High voltage cannot be used during the initial stages of IEF as movement of carrier ampholytes generate excessive heat.

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

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

Continued on next page

Materials Supplied by the User

The following reagents are needed to perform isoelectric focusing with Novex® IEF Gels. Ordering information for pre-mixed buffers is on page 63. If you are preparing your own buffers, recipes are provided on pages 68–69.

- Protein sample
- Deionized water
- IEF markers
- Novex®IEF Sample Buffer
- Novex®IEF Cathode Buffer
- Novex®IEF Anode Buffer
- Fixing solution

Preparing Anode Running Buffer (Lower Buffer Chamber)

Prepare 1X IEF Anode Buffer using Novex® IEF Anode Buffer (50X).

1. Prepare 1,000 mL of IEF Anode Buffer as follows:

Reagent	Amount
Novex® IEF Anode Buffer (50X)	20 mL
Deionized Water	980 mL
Total Volume	1,000 mL

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

Preparing Cathode Running Buffer (Upper Buffer Chamber)

Prepare 1X IEF Cathode Buffer using the appropriate Novex® IEF Cathode Buffer pH 3–10 (10X) or pH 3–7 (10X)

1. Prepare 200 mL of IEF Cathode Buffer as follows:

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

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

Preparing Sample

Samples for IEF Gels are prepared without SDS to avoid affecting the pI of the protein. Reducing agents are also **not recommended** for the same reason.

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. **Do not heat samples for IEF Gels.**

Continued on next page

IEF Gels, Continued

Electrophoresis Conditions

See page 32 for instructions on running Novex® Pre-Cast Gels using the XCell *SureLock*™ Mini-Cell. Run the gel at 100 V constant for 1 hour, followed by 200 V constant for 1 hour, and finish with 500 V constant for 30 minutes. See page 33 for additional details on electrophoresis conditions.

Fixing the Gel

Fixing the proteins in the IEF gel is recommended after electrophoresis. The fixing step also helps to remove carrier ampholytes from the gel, resulting in lower background after staining.

Fixing solution consists of 12% TCA, or 12% TCA wtih 3.5% sulfosalicylic acid.

1. Prepare 500 mL of fixing solution as follows:

Reagent	Amount
Trichloroacetic Acid (TCA)	60.0 g
Sulfosalicylic Acid (optional)	17.5 g
Deionized Water	to 500 mL
Total Volume	500 mL

2. Mix solution thoroughly.
3. Fix gels for 30 minutes.

Staining IEF Gels

IEF gels can be stained by Coomassie or colloidal blue techniques, refer to pages 35–38.

If using the SimplyBlue™ SafeStain, wash the gel extensively to remove traces of TCA from the fixation process to avoid formation of precipitate in the gel.

2D SDS-PAGE with IEF Gels

After staining the gel and documenting the results, proteins separated by pI can be separated by mass.

We recommend using NuPAGE® Bis-Tris or Novex® Tris-Glycine Gels with a 2D-well, or ZOOM® Gels for 2D SDS-PAGE.

2D-wells can fit strips of 6.5 cm, while ZOOM® IPG-wells can fit strips of 7 cm.

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

Continued on next page

Materials Supplied by the User

In addition to the appropriate gel with a 2D-well or IPG-well, the following reagents are needed to perform 2D gel electrophoresis with Novex® Gels.

- 20% Ethanol
- Sample Buffer (depending on your gel type)
- Running Buffer (depending on your gel type)
- Filter Paper
- NuPAGE® Sample Reducing Agent (optional)
- Iodoacetamide (optional)

Equilibrating the Gel

The SDS in the sample buffer and running buffer for SDS-PAGE strips the stain from proteins and resolubilizes the proteins for migration during 2D electrophoresis.

1. Incubate the IEF gel in 100 mL 20% ethanol for 10 minutes.
2. Cut out the desired lane (strip) from the IEF gel for SDS-PAGE.
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 with 1X Running Buffer.
4. Proceed

Optional Procedure for Reduced Samples:

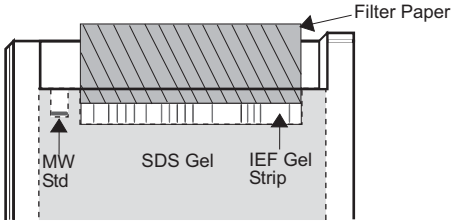
1. Incubate the IEF gel in 100 mL 20% ethanol for 10 minutes.
2. Cut out the desired lane (strip) from the IEF gel for SDS-PAGE.
3. Incubate the strip in 2 mL 2X SDS sample buffer and 0.5 mL ethanol for 3–5 minutes. Aspirate the sample buffer and rinse with 1X Running Buffer.
4. Prepare Reducing Solution by diluting 250 µL of the NuPAGE® Sample Reducing Agent (10X) in 1.75 mL of 1X SDS Sample Buffer.
5. Incubate the strip in Reducing Solution for 3–5 minutes. Decant the Reducing Solution.
6. Prepare 125 mM Alkylating Solution by adding 58 mg of fresh iodoacetamide to 2.5 mL of 1X SDS Sample Buffer.
7. Incubate the strip in Reducing Solution for 3–5 minutes.
8. Decant the Alkylating Solution and proceed to **2D Separation of Proteins on Novex® IEF Gels** (next page).

2D Separation of Proteins on Novex® IEF Gels

A protocol for separating proteins in an IEF gel strip by SDS-PAGE with the XCell *SureLock*™ Mini-Cell is provided below.

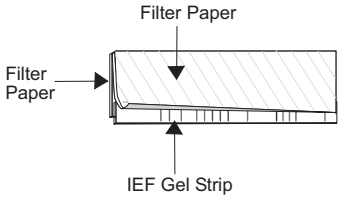
1. Fill the 2D or IPG-well with the appropriate 1X SDS Running Buffer.
2. Trim the IEF strip to a length of 5.8–5.9 cm (for 2D-wells) or 6.3–6.4 cm (for ZOOM® IPG-wells) such that the strip includes the pH regions containing your proteins of interest.
3. Transfer the IEF gel strip into the well of a 1.0 mm or 1.5 mm gel cassette as follows:
   - **For 1.0 mm Thick Gels**

     Slide the strip into the well using a gel-loading tip. Avoid trapping air-bubbles between the gel strip and the surface of the gel. Wet a piece of thick filter paper (5.8 × 4 cm) in 1X SDS Running Buffer and use it to push the IEF gel strip down so it makes contact with the surface of the gel (see figure below). The paper should hold the IEF gel strip in place.



   - **For 1.5 mm Thick Gels**

     Wet two pieces of thin filter paper (5.8 × 4 cm) in 1X SDS Running Buffer. Sandwich and the IEF gel strip with the filter paper, such that the edge of the gel strip protrudes ~0.5 mm beyond the edge of the paper (see figure below). Insert the sandwich into the well and push the strip so it comes in contact with the gel. Avoid trapping air-bubbles between the gel strip and the surface of the gel.



Electrophoresis Conditions

See page 32 for instructions on running Novex® Pre-Cast Gels using the XCell *SureLock*™ Mini-Cell.

Run the gel at 125 V constant. After the dye front has moved into the stacking gel (~10 min), disconnect the power supply, remove the filter paper, and resume electrophoresis to completion.

Staining the Gel

Stain the gel with the appropriate method for the type of gel and sample amount after electrophoresis. Refer to the techniques described on pages 35–46.

ZOOM® Gels

ZOOM® Gels

ZOOM® Gels are used for 2D analysis of proteins following isoelectric focusing of IPG strips. ZOOM® Gels are 1.0 mm thick, and 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 63 for ordering information)

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

2D Separation of IPG Strips

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. For 2D separation of Novex® IEF Gel strips, see page 21.

Materials Supplied by the User

You will need the following items for running ZOOM® Gels (see pages 63–64 for ordering information on Invitrogen products):

- 4X NuPAGE® LDS Sample Buffer
- NuPAGE® Sample Reducing Agent
- NuPAGE® Novex® 4–12% Bis-Tris ZOOM® Gel or Novex® 4–20% Tris-Glycine ZOOM® Gel
- Running Buffer (depending on your gel type)
- 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
- Protein molecular weight marker

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 116 mg of fresh iodoacetamide to 5 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 immediately to **SDS-PAGE**, page 25.

Continued on next page

SDS-PAGE

A protocol for separating proteins in an IPG strip by SDS-PAGE with ZOOM® Gels and the XCell *SureLock*™ Mini-Cell is provided below.

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 trapping air bubbles between the strip and the gel while sliding the strip into the well.
6. Pour ~ 400 µL of 0.5% agarose solution into the ZOOM® Gel well to seal the IPG strip in place. Make sure the agarose solution does not overflow into the molecular weight marker well.

Electrophoresis Conditions

See page 32 for instructions on running Novex® Pre-Cast Gels using the XCell *SureLock*™ Mini-Cell.

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.

Perform electrophoresis at 200 V for 40 minutes for NuPAGE® Novex® Bis-Tris ZOOM® Gels or at 125 V for 90 minutes for Novex® Tris-Glycine ZOOM® Gels.

Staining the Gel

Stain the gel with the appropriate method for the type of gel and sample amount after electrophoresis. Refer to the techniques described on pages 35–46.

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 10–3,000 base pairs.

Advantages of TBE Gels

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

The following reagents are needed to perform gel electrophoresis with Novex® TBE Gels. Ordering information for pre-mixed buffers is on page 63. If you are preparing your own buffers, recipes are provided on pages 69–70.

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

Preparing Running Buffer

Use 1X Novex® TBE Running Buffer to perform electrophoresis.

1. Prepare 1,000 mL of Running Buffer as follows:

Reagent	Amount
Novex® TBE Running Buffer (5X)	200 mL
Deionized Water	800 mL
Total Volume	1,000 mL

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

Continued on next page

Preparing Samples

Novex® TBE Gels require only ~10% of the amount of sample used on large gels or agarose gels. Dilute your standards and samples to ~ 0.01 OD (0.2 µg/band) to avoid overloading the gel.

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.

Electrophoresis Conditions

See page 32 instructions for running TBE Gel using the XCell *SureLock*™ Mini-Cell. Run the gel at 200 V constant. See page 33 for additional details on electrophoresis conditions.

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

Staining the Gel

Novex® TBE Gels can be stained by silver staining, ethidium bromide, and SYBR® Green staining techniques after electrophoresis. Refer to pages 39–47 for more information on these techniques.

TBE-Urea Gels

Introduction

Novex® denaturing polyacrylamide TBE-Urea Gels provide high resolution of short single-strand oligonucleotides. The 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

The following reagents are needed to perform gel electrophoresis with Novex® TBE-Urea Gels. Ordering information for pre-mixed buffers is on page 63. If you are preparing your own buffers, recipes are provided on pages 69–70.

- DNA or RNA sample
- Deionized water
- Appropriate DNA or RNA markers
- Novex® TBE-Urea Sample Buffer
- Novex® Prep TBE-Urea Sample Buffer (for preparative electrophoresis only)
- Novex® TBE Running Buffer

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

- 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
- Avoid using buffers with formamide on TBE-Urea polyacylamide gels as it will result in fuzzy bands

Preparing Running Buffer

Use 1X Novex® TBE Running Buffer to perform electrophoresis.

1. Prepare 1,000 mL of Running Buffer as follows:
2.

Reagent	**Amount**
Novex® TBE Running Buffer (5X)	200 mL
Deionized Water	800 mL
Total Volume	1,000 mL

3. Mix thoroughly. Use this buffer to fill the Upper and Lower Buffer Chamber of the XCell *SureLock*™ Mini-Cell for electrophoresis.
4. Flush wells of the gel several times with 1X TBE Running Buffer to remove urea from the wells prior to loading samples to obtain sharp bands.

Continued on next page

Preparing Samples

Novex® TBE-Urea Gels require only ~10% of the amount of sample used on large gels or agarose gels. Dilute your standards and samples to ~ 0.01 OD (0.2 µg/band) to avoid overloading the gel.

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. Heat samples at 70°C for 3 minutes to denature the samples.
3. Load the samples immediately on the gel. If the samples are not used immediately, place them on ice to prevent renaturation.

Electrophoresis Conditions

See page 32 instructions for running TBE-Urea Gel using the XCell *SureLock*™ Mini-Cell. Run the gel at 180 V constant. See page 33 for additional details on electrophoresis conditions.

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

Staining the Gel

Novex® TBE-Urea Gels can be stained by silver staining, ethidium bromide, and SYBR® Green staining techniques after electrophoresis. Refer to pages 35–48 for more information on these techniques.

DNA Retardation Gels

Gel-Shift Assay

Novex® DNA Retardation Gels consist of 6% polyacrylamide prepared with 0.5X 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 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. The 0.5X TBE buffer is sufficient for good electrophoretic separation yet low enough to promote DNA/ protein interactions.

Detection is performed with ethidium bromide staining of DNA or, for greater sensitivity, with radiolabeling the DNA or protein.

Materials Supplied by the User

The following reagents are needed to perform gel electrophoresis with Novex® DNA Retardation Gels. Ordering information for pre-mixed buffers is on page 63. If you are preparing your own buffers, recipes are provided on pages 69–70.

- DNA sample
- Deionized water
- Novex® Hi-Density TBE Sample Buffer
- Novex® TBE Running Buffer

Preparing Samples

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.

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.

Continued on next page

DNA Retardation Gels, Continued

Preparing Running Buffer

Prepare 1,000 mL of 0.5X Novex® TBE Running Buffer as follows:.

1.

Reagent	Amount
Novex® TBE Running Buffer (5X)	100 mL
Deionized Water	900 mL
Total Volume	1,000 mL

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

Electrophoresis Conditions

See page 32 instructions for running DNA Retardation Gels using the XCell *SureLock*™ Mini-Cell. Run the gel at 100 V constant. See page 33 for additional details on electrophoresis conditions.

Staining the Gel

Gel-shift assays use labeled (radioactive, fluorescent, biotin) DNA fragments for visualization of results. Use the appropriate technique to develop the image for the type of label you are using.

Electrophoresis of Novex® Pre-Cast Gels

Introduction

Instructions are provided below for electrophoresis of 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 website at www.invitrogen.com or contact Technical Support (see page 76).

For information on sample and buffer preparation for Novex® Pre-Cast Gels, see pages 10–31.

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. Gently pull the comb out of the cassette in one smooth motion.
4. Rinse the sample wells with the appropriate 1X 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 running just one gel, use the plastic Buffer Dam in place of the second gel cassette to form the Upper Buffer Chamber.
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 check the seal again.
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 8 for recommended loading volumes).
9. Load appropriate protein molecular weight markers (see page 64 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

Power Supply Settings for Novex® Pre-Cast Gels

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.

Opening Novex® Pre-Cast Gel Cassettes

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 two plastic plates that make up the cassette. 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 on the gel.
4. Carefully remove and discard the top plate, allowing the gel to rest on the bottom (slotted) plate.
5. If blotting, proceed to page 52 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 gel foot from the bottom of the gel. Hold the Gel Knife 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 foot. 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 gel foot off of the gel after fixing and staining, but before drying.
7. Fix and stain the gel as described on pages 35–48. For developing the Zymogram gel for enzyme activity, see page 17. For fixing IEF gels, see page 21.

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, and Coomassie R-250.

If you are using other types of Coomassie staining kits, follow the appropriate 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 59.

Materials Supplied by the User

You will need the following items for staining your gel (see page 63 for ordering information on Invitrogen products):

- Staining container
- Deionized water
- Orbital Shaker

For SimplyBlue™ SafeStain (see page 36)**:**

- SimplyBlue™ SafeStain
- *Optional*: 20% NaCl
- *Optional*: Microwave oven
- 12% Trichloroacetic acid (for IEF gels)

For Colloidal Blue Staining Kit (see page 37)**:**

- Colloidal Blue Staining Kit
- Methanol
- *Optional*: 20% Ammonium sulfate
- Fixing solutino (for IEF gels)

For Coomassie R-250 Staining (see page 38)**:**

- 0.1% Coomassie R-250 in 40% ethanol and 10% acetic acid
- Destaining Solution consisting of 10% ethanol and 7.5% acetic acid
- *Optional*: Microwave oven

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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 website at www.invitrogen.com or contact Technical Support (page 76).

For general use with 1.0 mm and 1.5 mm thick Tris-Glycine Gels, and 1.0 mm thick Tricine, Zymogram, and IEF Gels (8 cm × 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 Zymogram Gels with SimplyBlue™ SafeStain after renaturing and developing the gel for enzyme activity.

1. Fix IEF Gels 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 decreases at this point 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 49.

Continued on next page

Colloidal Blue Staining Kit Protocol

A brief staining protocol for staining Novex® Gels with the Colloidal Blue Staining Kit is provided below. For more details on the staining procedure, refer to the Manual (IM-6025). This manual is available on our website at www.invitrogen.com or contact Technical Support (see page 76).

1. Fix the IEF Gel in fixing solution as described on page 21. 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 49.

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.

Continued on next page

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 49.

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 63 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 64.

Materials Supplied by the User

You will need following items for silver staining your gel (see page 63 for ordering information on Invitrogen products):

- 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 clean 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 website at www.invitrogen.com or contact Technical Support (see page 76).

Use 100 mL of each solution for each 1.0 mm thick, 8 × 8 cm Novex® 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. Add 100 mL ultrapure water to the gel. Microwave at high power for 30 seconds. Remove the gel from the microwave and gently agitate it for 2 minutes at room temperature. Decant the water, and repeat the step one more time.
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 the end of development.
10. Wash the gel with 100 mL of ultrapure water for 10 minutes. For gel drying, see page 49.

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

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Preparing Solutions for SilverXpress® Silver Staining

Prepare the reagents as described below. If you are staining two gels, double the reagent volumes.

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

Methanol	100 mL
Acetic Acid	20 mL
Ultrapure water	to 200 mL

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

Sulphosalicylic acid	7 g
TCA	24 g
Ultrapure water	to 200 mL

- **Sensitizing solution**

Methanol	100 mL
Sensitizer	5 mL
Ultrapure water	to 200 mL

- **Staining solution**

Stainer A	5 mL
Stainer B	5 mL
Ultrapure water	90 mL

- **Developing Solution**

Developer	5 mL
Ultrapure water	95 mL

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SilverXpress® Silver Staining Protocol

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

For gel drying, see page 49.

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

SYPRO® Ruby Staining

Introduction

Instructions are provided below for a basic and rapid protocol for Novex® Pre-Cast Gels (Novex® Tris-glycine gels, Novex® Tricine gels, ZOOM® gels, and Novex® IEF gels) for the detection of proteins, including glycoproteins and phosphoproteins.

Advantages of SYPRO® Ruby Staining

SYPRO® Ruby provides the following advantages:

- Linear quantitation range of over three orders of magnitude
- Compatible with subsequent analysis of proteins by Edman based sequencing or mass spectrometry in 1D or 2D format
- Compatible with non-denaturing gels and IEF gels (basic protocol)

Molecular Weight Calibration

Guidelines and apparent molecular weight values for Novex® protein molecular weight standards are provided on page 64.

Materials Supplied by the User

You will need following items for silver staining your gel (see page 63 for ordering information on Invitrogen products):

- SYPRO® Ruby gel stain
- Staining containers, 1 per gel (see below for details)
- Reagent-grade methanol
- Reagent-grade glacial acetic acid
- Trichloroacetic acid (for IEF gels only)
- Ultrapure water (18 megohm-cm recommended)
- Rotary shaker
- Powder-free latex or vinyl gloves
- Microwave oven (700–1200 W) (optional)
- Water bath set at 80°C (optional)

General considerations for the protocol include the following:

- Perform all fixation, staining, and washing steps with continuous, gentle agitation (e.g., on an orbital shaker at 50 rpm).
- We recommend polypropylene or polycarbonate containers for staining. Glass dishes are not recommended. Staining containers should be meticulously clean to minimize contamination and other artifacts.
- For convenience, gels may be left in fix solution overnight or longer.
- For convenience, gels may be left in SYPRO® Ruby stain indefinitely without overstaining, although speckling artifacts tend to increase over time.
- As with any fluorescent stain, cover the gel container during staining and subsequent wash steps to exclude light.

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SYPRO® Ruby Staining, Continued

Preparing Solutions for SYPRO® Ruby Staining

Prepare the reagents as described below. If you are staining two gels, double the reagent volumes. Increase volumes 1.5-fold for 1.5mm thick gels.

- **Fix Solution**

Methanol	100 mL
Glacial Acetic Acid	14 mL
Ultrapure water	to 200 mL

- **Fix Solution** for IEF Gels

Methanol	40 mL
Trichloroacetic Acid	10 g
Ultrapure water	to 100 mL

- **Wash Solution**

Methanol	10 mL
Glacial Acetic Acid	7 mL
Ultrapure water	to 100 mL

SYPRO® Ruby Basic Protocol

The basic protocol results in the maximum signal strength and widest linear dynamic range for staining of denaturing gels, non-denaturing gels, and IEF gels. Sensitivity is in the 1 ng range for most proteins.

1. After electrophoresis, place the gel into a clean container with 100 mL of Fix Solution and agitate on an orbital shaker for 30 minutes. Pour off the used fix solution and repeat once more with fresh Fix Solution.

   Note: For IEF Gels, place the gel into a clean container with 100 mL of IEF Fix Solution and agitate on an orbital shaker for 3 hours. After fixing, perform 3 washes in ultrapure water for 10 minutes each, before proceeding to the staining step.
2. Pour off the used fix solution.
3. Add 60 mL of SYPRO® Ruby gel stain to the tray containing the gel. Agitate on an orbital shaker overnight.
4. Transfer the gel to a clean container and wash in 100 mL of Wash Solution for 30 minutes. The transfer step helps minimize background staining irregularities and stain speckles on the gel.
5. Rinse the gel in ultrapure water for 5 minutes. Repeat the rinse a minimum of one more time to prevent possible corrosive damage to your imager.

Note: If you are staining two gels, double the reagent volumes. Increase volumes 1.5-fold for 1.5mm thick gels.

Visualization of SYPRO® Ruby Stained Gels

Proteins stained with SYPRO® Ruby protein gel stain are readily visualized using a UV or blue-light source. The use of a photographic camera or CCD camera and the appropriate filters is essential to obtain the greatest sensitivity.

Continued on next page

Using SYPRO® Ruby Stain as a Post-Stain

SYPRO® Ruby stain can be used to post-stain gels stained with other gel stains such as Pro-Q® Diamond phosphoprotein gel stain, Pro-Q®Emerald 300 glycoprotein gel stain, Pro-Q® Sapphire or InVision™ oligohistidine-tag gel stains, or Pro-Q® Amber transmembrane protein gel stain.

Always use SYPRO® Ruby stain last, as the SYPRO® Ruby signal can dominate the signal from other stains. SYPRO® Ruby stain does not work well as a post-stain for colorimetric stains such as Coomassie and silver stains.

SYBR® Green Staining

Introduction

The SYBR® Green I nucleic acid gel stain is a sensitive stain that can be used to detect DNA in Novex® TBE and TBE-Urea Gels. As little as 20–60 pg of double stranded DNA, 100–300 pg of single stranded DNA, or 1–2 ng of a synthetic 24-mer can be detected, depending upon the wavelength of transillumination.

General considerations for the protocol include the following:

- We recommend polypropylene containers for staining. Glass dishes are not recommended. Staining containers should be meticulously clean to minimize contamination and other artifacts.
- SYBR® Green I reagent has optimal sensitivity at pH 7.5–8.0.
- For convenience, gels may be left in SYBR® Green I stain for up to 24 hours with little decrease in sensitivity.

Procedure

Perform post-staining of DNA on TBE or TBE-Urea Gels as follows:

1. Prepare a 1:10,000 dilution of SYBR® Green I reagent in TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0), TBE, or TAE buffer.
2. Remove the gel from the cassette using a Gel Knife, and place it in a polypropylene staining container.
3. Cover the gel with staining solution and incubate at room temperature for 10–40 minutes with gentle agitation. Protect the staining container from light by covering it with aluminum foil.

Visualization of SYBR® Green I Stained Gels

SYBR® Green I stain is compatible with a wide variety of gel reading instruments, ranging from ultraviolet transilluminators to argon laser and mercury-arc lamp excitation gel scanners. SYBR® Green I stain is maximally excited at 497 nm, but also has secondary excitation peaks at ~290 nm and ~380 nm. The fluorescence emission of SYBR® Green I stain bound to DNA is centered at 520 nm.

Ethidium Bromide Staining

Introduction

A brief protocol is provided below for staining nucleic acids on TBE and TBE-Urea Gels with ethidium bromide.

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. Remove the gel from the cassette using a Gel Knife, and place it in a staining container.
3. Incubate the gel in the ethidium bromide solution for 20 minutes.
4. Destain the gel by rinsing the gel three times with ultrapure water for 10 minutes.

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.

For alternative techniques with greater detection sensitivity, perform silver staining using the SilverXpress® Silver Staining Kit (see page 42) or SYBR® Green I staining (see page 47).

Gel Drying

Introduction

Dry gels by 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 the DryEase® Mini-Gel Drying System (see below). For applications that require vacuum drying, follow the recommendations on page 51 to minimize cracking of the gels.

Materials Supplied by the User

You will need the following items for drying your gel (see page 63 for ordering information on Invitrogen products):

- DryEase® Mini-Gel Drying System
- Gel-Dry™ Drying Solution (or prepare your own gel drying solution containing 30% methanol and 5% glycerol)
- StainEase® Gel Staining Tray or a suitable 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 download from our website at www.invitrogen.com or contact Technical Support (see page 76).

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.

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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 takes 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 so they remain flat for scanning, photography, display, and overhead projection.

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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 34. 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 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. Make sure the vacuum pump is in working condition, and properly set up to form a tight seal when on. Use drying conditions for polyacrylamide gels, with the temperature increasing to a set value and holding for the duration of the drying cycle. We recommend drying mini-gels at 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 residual moisture in the tubing and additional drying time is required.

Blotting Novex® Pre-Cast Gels

Introduction

After performing electrophoresis, proteins can be transferred to membranes for subsequent analysis. Methods of transfer include wet, semi-wet, semi-dry, and dry blotting. Semi-dry blotting can be performed with the Novex® Semi-Dry Blotter, and dry blotting is performed with the iBlot® Gel Transfer Device. Refer to the respective manuals for information on blotting with these devices.

Instructions are provided below for semi-wet blotting of Novex® Pre-Cast Gels using the XCell II™ Blot Module. For more information on the XCell II™ Blot Module, refer to the manual (IM-9051) available at www.invitrogen.com or contact Technical Support (see page 76).

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

In addition to the XCell II™ Blot Module, the following reagents are needed for blotting your gel (see page 63 for ordering information on Invitrogen products):

- Blotting membranes
- Filter paper (not needed if using Novex® pre-cut membrane/filter paper sandwiches)
- Methanol (if using PVDF membranes)
- Appropriate Transfer Buffer
- Deionized water

Preparing Transfer Buffer

For blotting Tris-Glycine, Tricine, and IEF Gels use 1X Tris-Glycine Transfer Buffer. If you are preparing your own transfer buffer see page 66 for a recipe.

An alternate transfer protocol for IEF Gels is provided on page 57.

If you are performing protein sequencing, an alternate transfer buffer compatible with the technique is listed on the next page.

Prepare 1,000 mL of Transfer Buffer:

Tris-Glycine Transfer Buffer (25X)	40 mL
Methanol	200 mL
Deionized Water	760 mL
Total Volume	1,000 mL

Continued on next page

Blotting Novex® Pre-Cast Gels, Continued

Preparing Transfer Buffer for TBE Gels

For blotting TBE and TBE-Urea Gels, use 0.5X TBE Running Buffer. If you are preparing your own transfer buffer, see page 69 for a recipe.

Prepare 1,000 mL of 1X Tris-Glycine Transfer Buffer using the Tris-Glycine Transfer Buffer (25X) as follows:

TBE Running Buffer (5X)	40 mL
Methanol	200 mL
Deionized Water	760 mL
Total Volume	1,000 mL

For blotting TBE and TBE-Urea Gels

Dilute the 5X TBE Running Buffer to 0.5X with deionized water.

Preparing Transfer Buffer Compatible with Protein Sequencing

Tris-Glycine Transfer Buffer interferes 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 Paper

Cut the transfer membrane and filter paper to the dimensions of the gel or use Novex® pre-cut membrane/filter paper sandwiches.

- **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 34, remove wells with the Gel Knife.
2. Place a piece of pre-soaked filter paper on top of the gel, with the edge 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 separate the gel from the plate.
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 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 it on the pad such that the gel is closest to the cathode plate (see Figure 1, next page).
9. Add enough pre-soaked blotting pads to raise the assembly 0.5 cm over the edge 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 fits into the unit only one way, with the (+) sign at the upper left hand corner of the blot module, and the inverted gold post fitting into the connector 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 Transfer Buffer. To avoid generating extra conductivity and heat, **do not** fill the chamber all the way to the top.
14. Fill the Lower 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 1–7 (previous page) twice to make two gel-membrane assemblies.
2. Place two pre-soaked pads on cathode shell of blot module. Place the first gel/membrane assembly on the pads such that the gel faces the cathode plate. (See Figure 2).
3. Add another pre-soaked blotting pad on top of first gel/membrane assembly.
4. Position second gel/membrane assembly on top of blotting pad with the gel facing 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

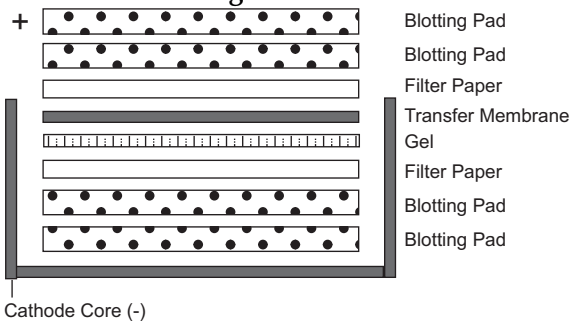
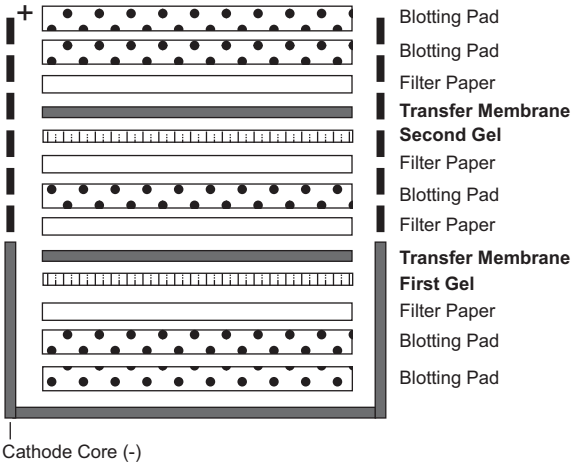


Figure 2



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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 is roughly twice the listed value.

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 Novex® Pre-Cast Gels, Continued

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. Prepare chilled 0.7% acetic acid.
2. After electrophoresis, remove the gel from the cassette and equilibrate the gel in the 0.7% acetic acid for 10 minutes.

   Tip: The 5% polyacrylamide gels are stickier and more difficult to handle than higher percentage polyacrylamide gels. To prevent the gel from sticking to the filer paper before it is in the proper position, remove the gel from the equilibration solution by submerging a piece of filter paper under the gel while it is floating in the equilibration solution. When the gel and filter paper are in the correct position, lift the filter paper so that it attaches to the gel.
3. Assemble the gel/membrane sandwich as described on page 54, except in **reverse order** so that the membrane is on the cathode (–) side of the gel.
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

Values for apparent molecular weight of Novex® molecular weight standards are 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.

Novex® Sharp Pre-stained Protein Standard	Tris-Glycine Gels (4–20%)	Tricine Gels (10–20%)
Band 1	260 kDa	260 kDa
Band 2	160 kDa	160 kDa
Band 3	110 kDa	110 kDa
Band 4	80 kDa	80 kDa
Band 5	60 kDa	60 kDa
Band 6	50 kDa	50 kDa
Band 7	40 kDa	40 kDa
Band 8	30 kDa	30 kDa
Band 9	20 kDa	20 kDa
Band 10	15 kDa	15 kDa
Band 11	10 kDa	10 kDa
Band 12		3.5 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.

Observation	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	• Load the appropriate amount of protein as described on page 8.
	• High salt concentration in the sample	• Decrease the salt concentration of your sample using dialysis or gel filtration
	• Sample precipitates	• Increase the concentration of SDS in your sample if necessary, to maintain the solubility of the protein.
	• Contaminants such as membranes or DNA complexes in the sample	• Centrifuge or clarify your sample to remove particulate contaminants
Bands in the outer lane of the gel are curving upwards	• Concentrated buffer used	• The pre-made buffers are supplied as concentrate. Dilute the buffers as described in this manual.
	• Expired gels used	• Avoid using gels after the expiration date.
	• High voltage used	• Electrophorese the gel using conditions described on page 33.

Continued on next page

Observation	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 10.
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 8. 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 website at www.invitrogen.com or call Technical Support (see page 76).

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
iBlot® Gel Transfer Device	1 unit	IB1001
Novex® Semi-Dry Blotter	1 unit	SD1000
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
Novex® pH 3-7 IEF Buffer Kit (includes LC5300, LC5370, LC5371)	1 kit	LC5377
Novex® pH 3-10 IEF Buffer Kit (includes LC5300, LC5310, LC5311)	1 kit	LC5317
UltraPure™ Agarose	100 g	15510-019
Nitrocellulose (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 website at www.invitrogen.com or contact Technical Support (see page 76).

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
Novex® Sharp Pre-stained Protein Standard	For visualizing protein molecular weight range and evaluating transfer efficiency	2 × 250 µL	LC5800
BenchMark™ Protein Ladder	For estimating the apparent molecular weight of proteins	2 × 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 website at www.invitrogen.com or contact Technical Support (see page 76).

Recipes

Tris-Glycine SDS Running Buffer

The Tris-Glycine SDS Running Buffer is available from Invitrogen (see page 63).

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

1. To prepare 1,000 mL of 10X 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 1,000 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 12). 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 63).

25 mM Tris base
192 mM Glycine
pH 8.3

1. To prepare 1,000 mL of 10X 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 1,000 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 12). 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 63).

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 63).

1X composition
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 63).

12 mM Tris Base
96 mM Glycine
pH 8.3

1. To prepare 500 mL of 25 × 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 52. 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 63).

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 63).

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

1. To prepare 1,000 mL of 10 × 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 1,000 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 15). The pH of the 1X solution is 8.3. Do not use acid or base to adjust the pH.

10X Zymogram Renaturing Buffer

The Zymogram Renaturing Buffer is available from Invitrogen (see page 63).

25% (v/v) Triton® X-100

1. To prepare 500 mL of 10X Zymogram Renaturing Buffer, add 125 mL Triton® X-100 to 300 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 63).

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	1.0 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 18).

IEF Sample Buffer pH 3–7

The IEF Sample Buffer pH 3–7 is available from Invitrogen (see page 63).

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 63).

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

1. To prepare 10 mL of 2X IEF Sample Buffer pH 3–10, 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 63).

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 63).

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 63).

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 63).

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

1. To prepare 1,000 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 1,000 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 26. 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 63).

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 63).

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 63).

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:

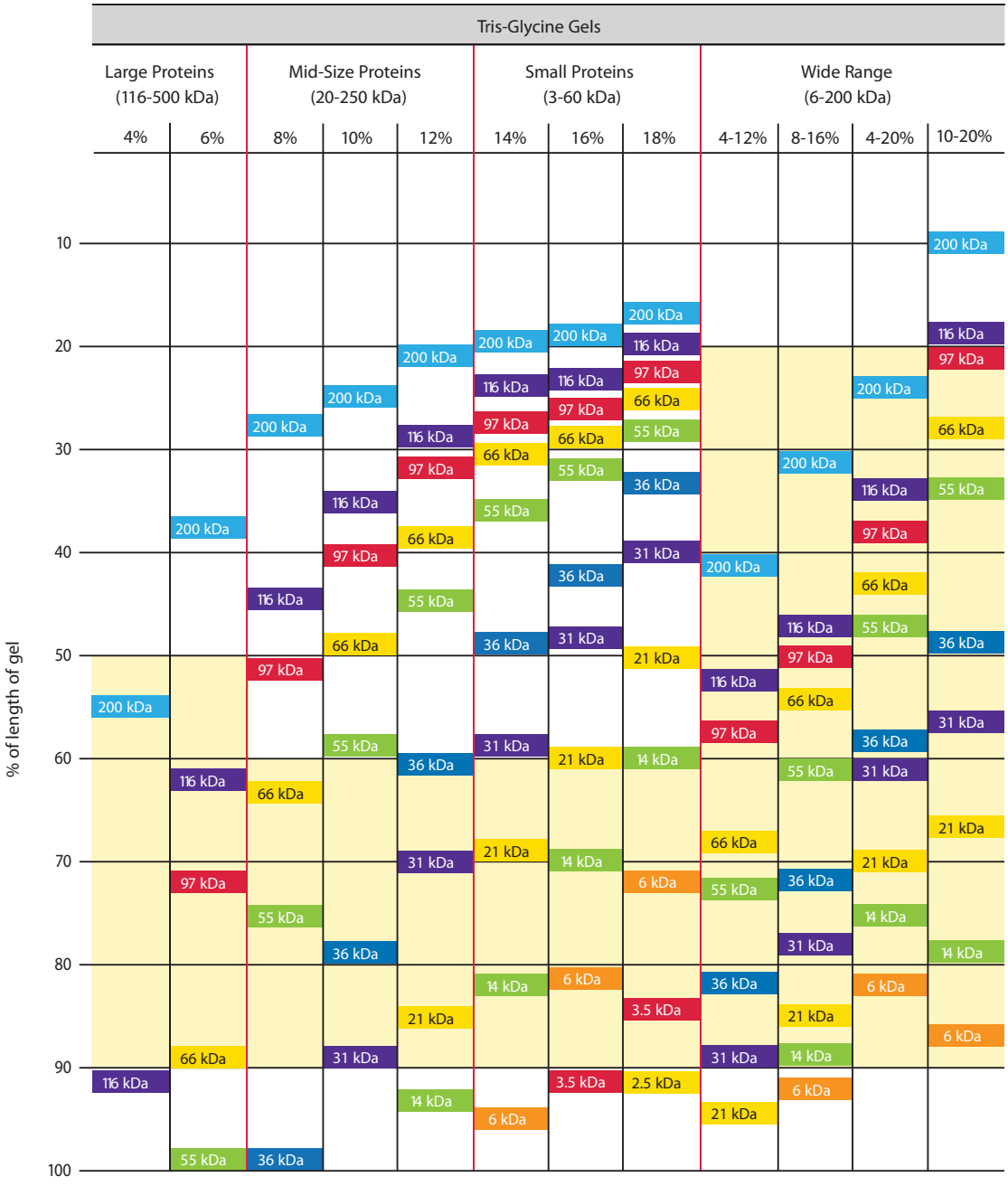
5X TBE Running Buffer (see page 69)	2 mL
Ficoll® Type 400	1.2 g
Urea	4.2 g

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.

Gel Migration Charts

Novex® Tris-Glycine Gel Migration Chart

The migration patterns of protein standards* on Novex® Tris-Glycine Gels are shown on the table below. Use the table to select the proper gel for separating proteins based on size. Optimal resolution is achieved when protein bands migrate within the shaded regions.



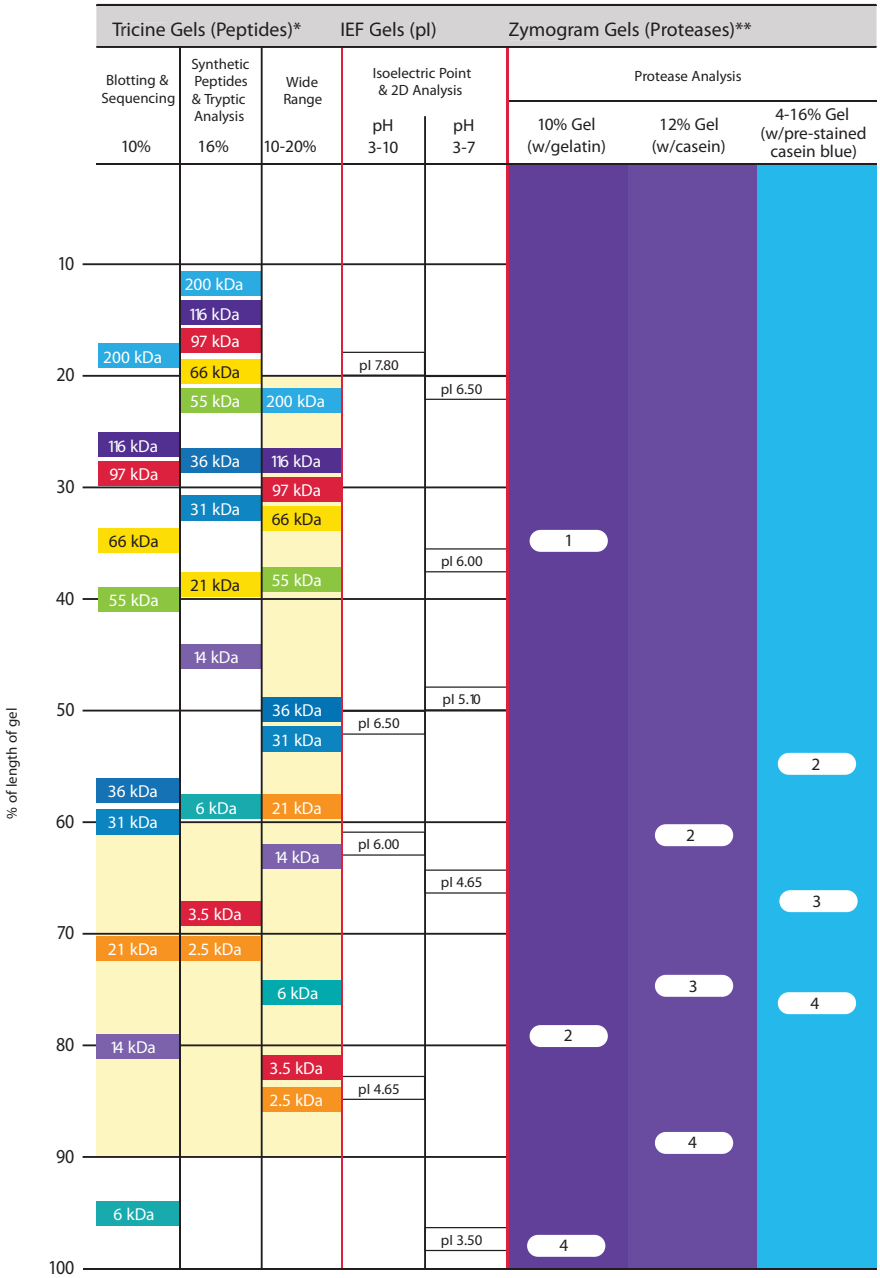
* Bands correspond to the migration of Mark12™ Unstained Standard under denaturing conditions.

Continued on next page

Gel Migration Charts, Continued

Novex® Tricine, IEF, and Zymogram Gel Migration Chart

The migration patterns of protein markers on Novex® Tricine, IEF, and Zymogram Gels are shown on the table below. Optimal resolution is achieved when protein bands migrate within the shaded regions.



* Bands correspond to the migration of Mark12™ Unstained Standard under denaturing conditions.

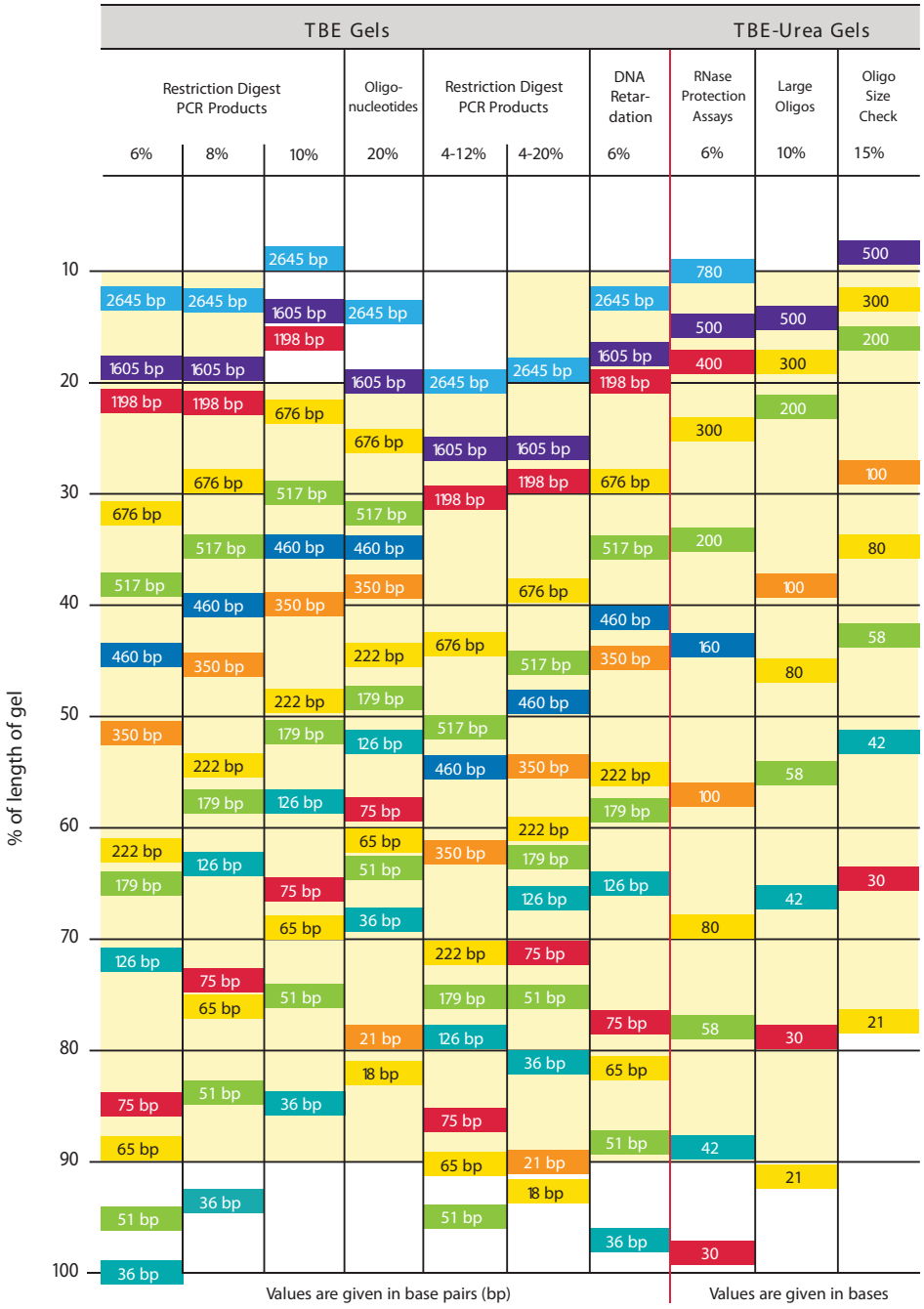
** The numbered bands on the Zymogram Gel patterns refer to the following proteases: Band 1: Collagenase Type I (140 kDa); Band 2: Thermolysin (37 kDa); Band 3: Chymotrypsin (30 kDa); Band 4: Trypsin (19 kDa)

Continued on next page

Gel Migration Charts, Continued

Novex® TBE and TBE-Urea Gel Migration Chart

The migration patterns of DNA fragments on Novex® TBE and TBE-Urea Gels are shown on the table below. Optimal resolution is achieved when nucleic acid bands migrate within the shaded regions.



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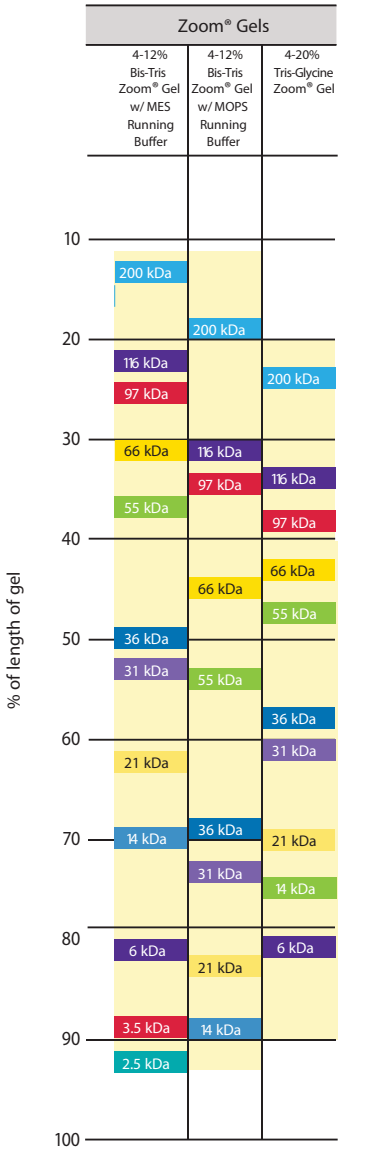
Gel Migration Charts, Continued

ZOOM® Gel Migration Chart

The migration patterns of protein standards* on ZOOM® Gels are shown on the table below. Optimal resolution is achieved when protein bands migrate within the shaded regions.



* On ZOOM® Gels, migration of bands correspond to the migration of Mark12™ Unstained Standard (Cat. no. LC5677) under denaturing conditions.

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