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Instruction Manual

NuPAGE® Technical Guide

General information and protocols for using the NuPAGE® electrophoresis system

Version E
October 1, 2003
IM-1001

Table of Contents

General Information

Purpose of the Guide

The NuPAGE® Technical Guide contains information about the NuPAGE® Electrophoresis System and is intended to supplement the NuPAGE® Bis-Tris Gel Instruction Card (IM-8042) and the NuPAGE® Tris-Acetate Gel Instruction Card (IM-1025). Complete protocols for sample preparation, buffer preparation, electrophoresis, staining, and blotting are provided in this guide.

To request the Instruction Cards or for additional information, contact Technical Service (see page 51) or you may download the manuals from our web site at www.invitrogen.com.

For description of the NuPAGE® electrophoresis system, see page 7.

Storage and Shelf life

Store NuPAGE® Novex Bis-Tris Gels at 4-25°C and NuPAGE® Novex Tris-Acetate Gels at +4°C.

The NuPAGE® Novex Bis-Tris Gels have a shelf life of 12 months when stored at 4-25°C.

The NuPAGE® Novex Tris-Acetate Gels have a shelf life of 8 months when stored at 4°C.

Do not freeze NuPAGE® Gels.

Using expired gels or improperly stored gels may result in poor band resolution.

Handling the Gels

Gels are individually packaged in clear pouches with 10 ml of Packaging Buffer. The Packaging Buffer contains low levels of residual acrylamide monomer and 0.02% sodium azide. Gloves should be worn at all time when handling gels.

Warning: This product contains a chemical (acrylamide) known to the state of California to cause cancer. To obtain a MSDS, see page 51.

NuPAGE® Gel Specifications

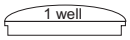
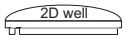
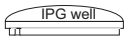
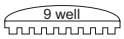
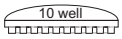
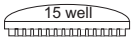
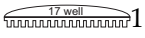
Specifications

Gel Matrix:	Acrylamide/Bisacrylamide
Gel Thickness:	1.0 mm
Gel Size:	8 cm x 8 cm
Cassette Size:	10 cm x 10 cm
Cassette Material:	Styrene Copolymer (recycle code 7)
Sample Well Configuration:	1, 9, 10, 12, 15, 17-well, 2D-well, and IPG well

Recommended Loading Volumes

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

Note: The 9- and 17-wells are compatible with any eight-channel pipette 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	Silver Staining	Immunoblotting
 1.0 mm	700 µl	12 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.	Scale your sample load according to the sensitivity of your detection method.
 1.0 mm 1.5 mm	400 µl 600 µl	12 µg/band		
 1.0 mm	7 cm IPG Strip	N/A		
 1.0 mm	28 µl	0.5 µg/band		
 1.0 mm 1.5 mm	25 µl 37 µl	0.5 µg/band		
 1.0 mm	20 µl	0.5 µg/band		
 1.0 mm 1.5 mm	15 µl 25 µl	0.5 µg/band		
 1.0 mm	15 µl	0.5 µg/band		

Description of the NuPAGE® Electrophoresis System

Introduction

The NuPAGE® Bis-Tris Electrophoresis System is a revolutionary neutral pH, discontinuous SDS-PAGE, pre-cast polyacrylamide mini-gel system. The neutral pH 7.0 environment during electrophoresis results in maximum stability of both proteins and gel matrix, providing better band resolution than other gel systems (see **Advantages of the NuPAGE® Electrophoresis System,** below).

The Laemmli system is the most widely used SDS-PAGE method for separating a broad range of proteins (Laemmli, 1970). The highly alkaline operating pH of the Laemmli system may cause band distortion, loss of resolution, or artifact bands. The major causes of poor band resolution with the Laemmli system are:

- Hydrolysis of polyacrylamide at the high gel casting pH of 8.7 resulting in a short shelf life of 4-6 weeks
- Chemical modifications such as deamination and alkylation of proteins due to the high pH (9.5) of the separating gel
- Reoxidation of reduced disulfides from cysteine containing proteins as the redox state of the gel is not constant
- Cleavage of Asp-Pro bond of the proteins when heated at 100°C in the Laemmli sample buffer, pH 5.2 (Kubo, 1995).

Advantages of the NuPAGE® Electrophoresis System

The operating neutral pH of the NuPAGE® Gels and buffers provide following advantages over the Laemmli system:

- Longer shelf life of 8-12 months due to improved gel stability (see page 5)
- Improved protein stability during electrophoresis at neutral pH resulting in sharper band resolution and accurate results (Moos *et al*, 1998)
- Complete reduction of disulfides under mild heating conditions (70°C for 10 minutes) and absence of cleavage of asp-pro bonds using the NuPAGE® LDS Sample buffer (pH > 7.0 at 70°C)
- Reduced state of the proteins maintained during electrophoresis and blotting of the proteins by the NuPAGE® Antioxidant

NuPAGE® Electrophoresis System Components

The NuPAGE® Electrophoresis System consists of:

- NuPAGE® Novex Bis-Tris [Bis (2-hydroxyethyl) imino-tris (hydroxymethyl) methane-HCl] Pre-Cast Gels for separating small to mid-size molecular weight proteins
- NuPAGE® Novex Tris-Acetate Pre-Cast Gels for separating large molecular weight proteins
- NuPAGE® LDS (Lithium dodecyl sulfate) Sample Buffer
- NuPAGE® Reducing Agent
- NuPAGE® Antioxidant
- NuPAGE® MES [2-(N-morpholino) ethane sulfonic acid] SDS or MOPS [3-(N-morpholino) propane sulfonic acid] SDS Running Buffer for NuPAGE® Novex Bis-Tris Gels
- NuPAGE® Tris-Acetate SDS Running Buffer for NuPAGE® Novex Tris- Acetate Gels
- NuPAGE® Transfer Buffer for blotting of NuPAGE® Novex Pre-Cast Gels

Continued on next page

Description of the NuPAGE® Electrophoresis System, Continued

NuPAGE® Bis-Tris Discontinuous Buffer System

The NuPAGE® Bis-Tris discontinuous buffer system involves three ions:

- Chloride ($^-$) is supplied by the gel buffer and serves as a leading ion due to its high affinity to the anode as compared to other anions in the system. The gel buffer ions are Bis-Tris$^+$ and Cl^- (pH 6.4).
- MES or MOPS ($^-$) serves as the trailing ion. The running buffer ions are Tris$^+$, MOPS$^-$/MES$^-$, and dodecylsulfate ($^-$) (pH 7.3-7.7).
- Bis-Tris ($^+$) is the common ion present in the gel buffer and running buffer. The combination of a lower pH gel buffer (pH 6.4) and running buffer (pH 7.3-7.7) results in a significantly lower operating pH of 7 during electrophoresis.

NuPAGE® Tris-Acetate Discontinuous Buffer System

The NuPAGE® Tris-Acetate discontinuous buffer system involves three ions:

- Acetate ($^-$) is supplied by the gel buffer and serves as a leading ion due to its high affinity to the anode as compared to other anions in the system. The gel buffer ions are Tris$^+$ and Acetate$^-$ (pH 7.0).
- Tricine ($^-$) serves as the trailing ion from the running buffer. The running buffer ions are Tris$^+$, Tricine$^-$, and dodecylsulfate ($^-$) (pH 8.3).
- Tris ($^+$) is the common ion present in the gel buffer and running buffer. The Tris-Acetate system also operates at a significantly lower operating pH of 8.1 during electrophoresis.

Separation Range of Proteins

The NuPAGE® Gels have a wider range of separation on a single gel and also separate proteins evenly throughout the low and high molecular weight ranges than existing gels. Due to these advantages, most proteins are well resolved on one of the five NuPAGE® gels (see **Types of NuPAGE® Gels**, next page).

By combining any of the NuPAGE® Novex Bis-Tris Gels with the MES SDS or MOPS SDS Running Buffer, you can obtain six separation ranges for resolving proteins over a wide molecular weight range of 1-200 kDa. The NuPAGE® Novex Tris-Acetate gels resolve proteins in the molecular weight range of 36-400 kDa.

To choose the correct NuPAGE® Gel for your application, refer to the Gel Migration Chart on our Web site at www.invitrogen.com or the catalog.

Continued on next page

Description of the NuPAGE® Electrophoresis System, Continued

Types of NuPAGE® Gels

The NuPAGE® Novex Pre-Cast Gels are available in different acrylamide concentrations, gel thickness, and well formats (see the table below).

NuPAGE® Novex Bis-Tris Gels	NuPAGE® Novex Tris-Acetate Gels
Separating Gel Acrylamide Concentration	
10%	3-8%
12%	7%
4-12%	
Stacking Gel Acrylamide Concentration	
4%	3.2%
Gel Thickness	
1.0 mm	1.0 mm
1.5 mm	1.5 mm
Well Format	
1, 9, 10, 12, 15, 17, 2D, and IPG well	10, 12, 15, and 2D well

Formulation

The formulation for the NuPAGE® Gels is listed below:

NuPAGE® Novex Bis-Tris Gels	NuPAGE® Novex Tris-Acetate Gels
Bis-Tris-HCl buffer (pH 6.4)	Tris base
Acrylamide	Acetic acid
Bis-acrylamide	Acrylamide
Ammonium persulfate (APS)	Bis-acrylamide
Ultrapure water	TEMED
The separating gel operates at pH 7.0.	Ammonium persulfate (APS)
	Ultrapure water
	The separating gel operates at pH 8.1

The NuPAGE® Gels do not contain SDS. However, they are designed for performing denaturing gel electrophoresis (see **Applications**, next page).

Crosslinker

The crosslinker concentration for the NuPAGE® Novex Pre-Cast Gel ranges from 3.8-5% depending on the region of the gel.

Compatibility

The size of a NuPAGE® Novex Pre-Cast Gel is 10 x 10 cm (gel size is 8 x 8 cm). We recommend using the XCell *SureLock*™ Mini-Cell (see page 45 for ordering information) for the electrophoresis of NuPAGE® Novex Pre-Cast Gels to obtain optimal and consistent performance.

Continued on next page

Description of the NuPAGE® Electrophoresis System, Continued

Staining NuPAGE® Gels

The NuPAGE® 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 20-23) for silver staining of NuPAGE® Gels.

The NuPAGE® Novex Pre-Cast Gels are compatible with any of the standard Coomassie staining procedures. The protocols that are accelerated by heat are preferable as the heat serves as a "fix" for proteins, especially smaller peptides. The SimplyBlue™ SafeStain and Novex® Colloidal Coomassie Blue Staining Kit (see pages 24-27) are recommended for staining NuPAGE® Gels.

The NuPAGE® Novex Pre-Cast Gels are also compatible with copper or zinc staining.

Applications

The NuPAGE® Novex Pre-Cast Gels are used:

- For separating proteins under denaturing conditions (NuPAGE® Bis-Tris Gels and NuPAGE® Tris-Acetate Gels)
- For separating proteins under non-denaturing (native) conditions (NuPAGE® Tris-Acetate Gels).
- For protein sequencing using Edman sequencing (from gels or PVDF)

Note: Do not use the NuPAGE® Bis-Tris Gels with NuPAGE® MOPS or MES Running Buffer without SDS for native gel electrophoresis. This buffer system may generate excessive heat resulting in poor band resolution. The protein of interest may not migrate very well in a neutral pH environment if it is not charged.

Preparing Samples

Introduction

General information on the sample buffer and reducing agent is provided below. For sample preparation protocols, see page 13.

NuPAGE® LDS Sample Buffer

Use the NuPAGE® LDS Sample Buffer (4X) for preparing samples for denaturing gel electrophoresis with the NuPAGE® Gels.

For native gel electrophoresis with NuPAGE® Tris-Acetate Gels, use the Novex® Tris-Glycine Native Sample Buffer (2X).

For optimal sample preparation in all SDS-PAGE protocols, including the NuPAGE® system, denature and reduce the protein disulfide bonds under slightly alkaline pH conditions. Since the pH of the NuPAGE® LDS Sample Buffer is 8.4, sample reduction at this pH allows for maximal activity of the reducing agent.

Note

The NuPAGE® LDS Sample Buffer is a 4X concentrated solution containing twice as much LDS as the 2X concentration of Novex® Tris-Glycine SDS or Tricine SDS Sample Buffer. This makes the NuPAGE® LDS Sample Buffer more viscous and difficult to pipet as compared to the Novex® Tris-Glycine or Tricine Buffers.

The presence of more glycerol also increases the viscosity of the NuPAGE® LDS Sample Buffer. By bringing the NuPAGE® LDS Sample Buffer to room temperature (25°C), the buffer is more manageable.

Tracking Dye

The NuPAGE® LDS Sample Buffer contains Coomassie G250 and Phenol Red as tracking dyes instead of bromophenol blue.

Coomassie G250 gives a sharp dye front with both MES and MOPS SDS Running Buffers and migrates much closer to the moving ion front than bromophenol blue. This ensures that small peptides do not run off the gel. Bromophenol blue runs more slowly than some peptides with the MES SDS Running Buffer.

The concentration of the tracking dye (Coomassie G250) is increased in the NuPAGE® LDS Sample Buffer to enhance viewing of the dye front.

Reducing Agent

The NuPAGE® Reducing Agent contains 500 mM dithiothreitol (DTT) at a 10X concentration and is available in a ready-to-use, stabilized liquid form (see page 45 for ordering information). Use the NuPAGE® Reducing Agent to prepare samples for reducing gel electrophoresis.

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

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

Continued on next page

Important

Do not use the NuPAGE® Antioxidant as a sample reducing agent. The antioxidant is not efficient in reducing the disulfide bonds. This will result in partially reduced bands with substantial background smearing in the lane.

The antioxidant maintains the sample proteins that have been previously reduced with a reducing agent in a reduced state and prevents the proteins from reoxidizing during electrophoresis.

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, follow these guidelines:

- 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.
- If you are running reduced and non-reduced samples on the same gel, omit the antioxidant (see page 14). The antioxidant will have a deleterious effect on the non-reduced samples. The bands will be sharper on NuPAGE® Gels relative to other gel systems, even without the use of the antioxidant.

Materials Supplied by the User

You will need the following items:

- Protein sample
- Deionized water

For denaturing electrophoresis

- NuPAGE® LDS Sample Buffer (see page 45 for ordering information or page 47 for a recipe)
- NuPAGE® Reducing Agent (see page 45 for ordering information)

For non-denaturing electrophoresis

- Novex® Tris-Glycine Native Sample Buffer (see page 45 for ordering information or page 47 for a recipe)

Continued on next page

Preparing Samples, Continued

Preparing Samples for Denaturing NuPAGE® Gel Electrophoresis

Instructions are provided below to prepare reduced or non-reduced samples for denaturing gel electrophoresis using the NuPAGE® Novex Bis-Tris or Tris-Acetate Gels.

The NuPAGE® LDS Sample Buffer and NuPAGE® Reducing Agent are available from Invitrogen (see page 45 for ordering information).

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

Reagent	Reduced Sample	Non-reduced Sample
Sample	x µl	x µl
NuPAGE® LDS Sample Buffer (4X)	2.5 µl	2.5 µl
NuPAGE® Reducing Agent (10X)	1 µl	--
Deionized Water	to 6.5 µl	to 7.5 µl
Total Volume	10 µl	10 µl

See page 47 for a recipe of sample buffer, if you are preparing the sample buffer.

Preparing Samples for Non-Denaturing NuPAGE® Gel Electrophoresis

Instructions are provided below to prepare samples for non-denaturing (native) gel electrophoresis using the NuPAGE® Novex Tris-Acetate Gels.

The Novex® Tris-Glycine Native Sample Buffer is available from Invitrogen (see page 45 for ordering information).

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

See page 47 for a recipe of the sample buffer, if you are preparing the sample buffer.

Heating Samples

Heat the sample for denaturing electrophoresis (reduced or non-reduced) at 70°C for 10 minutes for optimal results.

Do not heat samples for non-denaturing (native) electrophoresis.

Preparing Running Buffer

Introduction

General information on the running buffer and antioxidant is provided below. Instructions for preparing running buffers for denaturing and non-denaturing electrophoresis are provided on the next page.

NuPAGE® SDS Running Buffer

Three types of NuPAGE® Running Buffers are used for denaturing gel electrophoresis of NuPAGE® Gels. See page 45 for ordering information.

- NuPAGE® MES SDS Running Buffer is used with NuPAGE® Novex Bis-Tris Gels to resolve small molecular weight proteins
- NuPAGE® MOPS SDS Running Buffer is used with NuPAGE® Novex Bis-Tris Gels to resolve mid-size proteins
- NuPAGE® Tris-Acetate SDS Running Buffer is used with NuPAGE® Novex Tris-Acetate Gels to resolve high molecular weight proteins

The NuPAGE® MES SDS Running Buffer and NuPAGE® MOPS SDS Running Buffers have different pKa's, resulting in MES being a faster running buffer than MOPS. The difference in ion migration affects the stacking and the separation ranges of proteins with these buffers.

For native gel electrophoresis with NuPAGE® Novex Tris-Acetate Gels, use the Novex® Tris-Glycine Native Running Buffer.

To prepare Running Buffers for electrophoresis, see next page.

NuPAGE® Antioxidant

The reducing agents, DTT and β-mercaptoethanol, do not co-migrate through the gel with the sample in a neutral pH environment of the NuPAGE® Gels. Instead, the reducing agent tends to remain at the top of the gel and not migrate fully throughout the gel. Disulfide bonds are less reactive at neutral pH and are less likely to reoxidize than in a higher pH system. However, in the absence of an antioxidant some reoxidization may occur during the electrophoresis, resulting in slightly diffuse bands.

The NuPAGE® Antioxidant (a proprietary reagent) is added to the running buffer in the upper (cathode) buffer chamber only when performing electrophoresis under reducing conditions. The NuPAGE® Antioxidant migrates with the proteins during electrophoresis preventing the proteins from reoxidizing and maintaining the proteins in a reduced state. The NuPAGE® Antioxidant also protects sensitive amino acids such as methionine and tryptophan from oxidizing.

We also recommend using the NuPAGE® Antioxidant with reduced samples that have been alkylated, for optimal results.

The NuPAGE® Antioxidant is **NOT** compatible with gel systems other than the NuPAGE® system as the antioxidant is not efficient at higher pHs of other gel systems.

Continued on next page

Preparing Running Buffer, Continued

Materials Supplied by the User

You will need the following items:

- Deionized water

For denaturing electrophoresis

- NuPAGE® MES or MOPS SDS Running Buffer (see page 45 for ordering information or page 47 for a recipe)
- NuPAGE® Tris Acetate Running Buffer (see page 45 for ordering information or page 47 for a recipe)
- NuPAGE® Antioxidant

For non-denaturing electrophoresis

- Novex® Tris-Glycine Native Running Buffer (see page 45 for ordering information or page 47 for a recipe)

Preparing Buffer for Denaturing Electrophoresis

NuPAGE® SDS Running Buffer (20X) is available from Invitrogen (see page 45).

Reducing Conditions

1. Prepare 1000 ml of 1X NuPAGE® SDS Running Buffer using NuPAGE® SDS Running Buffer (20X) as follows:

NuPAGE® SDS Running Buffer (20X, MES, MOPS, or Tris-Acetate)	50 ml
Deionized Water	950 ml
Total Volume	1000 ml

2. Mix thoroughly and set aside 800 ml of the 1X NuPAGE® SDS Running Buffer for use in the Lower (Outer) Buffer Chamber of the XCell *SureLock*™ Mini-Cell.
3. Immediately, prior to electrophoresis, add 500 µl of NuPAGE® Antioxidant to 200 ml of 1X NuPAGE® SDS Running Buffer from Step 1 for use in the Upper (Inner) Buffer Chamber of the XCell *SureLock*™ Mini-Cell. Mix thoroughly.

Non-Reducing Conditions

1. Prepare 1000 ml of 1X NuPAGE® SDS Running Buffer using NuPAGE® SDS Running Buffer (20X) as follows:

NuPAGE® SDS Running Buffer (20X, MES or MOPS)	50 ml
Deionized Water	950 ml
Total Volume	1000 ml

2. Mix thoroughly. Fill the Upper and Lower Buffer Chamber of the XCell *SureLock*™ Mini-Cell with this Running Buffer.

See page 47 for a recipe of the NuPAGE® SDS Running Buffers, if you are preparing the running buffers.

Continued on next page

Preparing Running Buffer, Continued

Preparing Buffer for Non-Denaturing Electrophoresis

Novex® Tris-Glycine Native Running Buffer (10X) is available from Invitrogen (see page 45).

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

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

2. Mix thoroughly and use 800 ml of this Running Buffer in the Lower and Upper Buffer Chambers of the XCell *SureLock*™ Mini-Cell.

See page 47 for a recipe of the Novex® Tris-Glycine Native Running Buffer, if you are preparing the running buffer.

Note

- If you forget to add the antioxidant to the upper buffer chamber, some bands may be slightly fuzzier and more diffuse due to reoxidation of some proteins during electrophoresis.
- We recommend preparing the running buffer for the upper chamber with the antioxidant within half an hour of planned use. If the antioxidant is added to the running buffer too long before use, gels may exhibit signs of reoxidation (slightly fuzzier bands).
- If you have added 0.5 ml of antioxidant to the total amount of buffer (for upper and lower buffer chamber) by accident, the required concentration of antioxidant will be lower and the antioxidant will not be effective. If you wish to add antioxidant to the total amount of buffer (for upper and lower buffer chamber), add 2.5 ml of antioxidant to obtain the expected results. However, this is not recommended as high current will be generated and the antioxidant is wasted.
- If you have switched the antioxidant and the reducing agent (used the antioxidant in the sample buffer and the reducing agent in the running buffer) by accident, the reducing agent (DTT) will not migrate into the gel and the antioxidant will not effectively reduce the samples, resulting in decreased staining sensitivity.

Electrophoresis of NuPAGE® Gels

Introduction

Instructions are provided below for electrophoresis of the NuPAGE® Gels using the XCell *SureLock*™ Mini-Cell. For more information on the XCell *SureLock*™ Mini-Cell, refer to the manual (IM-9003). This manual is available on our Web site at www.invitrogen.com or contact Technical Service (see page 51).

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

Important

To ensure success with the NuPAGE® Electrophoresis System, remember the important points listed below:

- Under **NO** circumstances should Tris-Glycine SDS buffers be used with NuPAGE® Gels for any denaturing gel electrophoresis (see page 42 for the outcome of your results using incorrect buffers)
- Use **ONLY** NuPAGE® SDS buffers (see page 14)
- **DO NOT BOIL** samples. Heat samples at 70°C for 10 minutes (see page 13)
- Inner and Outer Buffer Chambers MUST be filled with the recommended amount of running buffer to prevent excessive heating (see below).

Procedure using XCell *SureLock*™ Mini-Cell

Wear protective gloves and safety glasses when handling gels.

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

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

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

6. Fill the Upper Buffer Chamber with a small amount of the running buffer to check for tightness of seal. If you detect a leak from Upper to the Lower Buffer Chamber, discard the buffer, reseal the chamber, and refill.
7. Once the seal is tight, fill the Upper Buffer Chamber (inner) with the appropriate 1X running buffer (see page 15 for running buffer preparation). The buffer level must exceed the level of the wells.

Continued on next page

Electrophoresis of NuPAGE® Gels, Continued

Procedure using XCell *SureLock*™ Mini-Cell, continued

Note: If you are running reduced samples, remember to fill the Upper Buffer Chamber with 200 ml of running buffer containing the NuPAGE® Antioxidant (see page 15).

8. Load an appropriate volume of sample at the desired protein concentration onto the gel (see page 6 for recommended loading volumes).
9. Load appropriate protein molecular weight markers (see page 45 for ordering information).
10. Fill the Lower (outer) Buffer Chamber with 600 ml of the appropriate 1X running buffer.

Electrophoresis Conditions

Run your gels according to the following protocol:

Gel Type	Voltage	Expected Current*	Run Time
NuPAGE® Novex Bis-Tris Gels with MES SDS Running Buffer	200 V constant†	Start: 110-125 mA/gel End: 70-80 mA/gel	35 minutes
NuPAGE® Novex Bis-Tris Gels with MOPS SDS Running Buffer	200 V constant†	Start: 100-115 mA/gel End: 60-70 mA/gel	50 minutes
NuPAGE® Novex Tris-Acetate Gels	150 V constant	Start: 40-55 mA/gel End: 25-40 mA/gel	1 hour
NuPAGE® Novex Tris-Acetate **Native** Gels	150 V constant	Start: 18 mA/gel End: 7 mA/gel	~2 hours Run times may vary

† Recommended voltage for 9 and 17-well gels is 150-175 volts

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Electrophoresis of NuPAGE® Gels, Continued

Removing the Gel after electrophoresis

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

   Caution: Use caution while inserting the gel knife between the two plates to avoid excessive pressure towards the gel.
4. Carefully remove and discard the top plate, allowing the gel to remain on the bottom (slotted) plate.
5. If blotting, proceed to the Western Transfer Protocol on page 31 without removing the gel from the bottom plate.
6. If staining, remove the gel from the plate by one of the methods:
   - Use the sharp edge of the gel knife to remove the bottom lip of the gel. The gel knife should be at a 90° angle, perpendicular to the gel and the slotted half of the cassette. Push down on the knife, and then repeat the motion across the gel to cut off the entire lip. Hold the plate and gel over a container with the gel facing downward and use the knife to carefully loosen one lower corner of the gel and allow the gel to peel away from the plate.
   - Hold the plate and gel over a container with the gel facing downward. Gently push the gel knife through the slot in the cassette, until the gel peels away from the plate. Cut the lip off of the gel after fixing, staining, but before drying.
7. Fix and stain the gel as described on pages 20-27.

Silver Staining

Introduction

Instructions are provided below for silver staining the NuPAGE® Gels using the SilverQuest™ Silver Staining Kit and the SilverXpress® Silver Staining Kit (see page 45 for ordering information).

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

The NuPAGE® system is more effective in reducing proteins and maintaining proteins in their reduced state. This may cause any minor contaminants present in the protein to be more visible under the sensitive silver staining techniques with the NuPAGE® system than in other systems.

Materials Supplied by the User

You will need following items for silver staining:

- 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; see page 45 for ordering information)

For SilverQuest™ Silver Staining

- Ethanol
- Fixative (40% ethanol, 10% acetic acid)

For SilverXpress® Silver Staining

- Methanol
- Acetic acid

For optimal silver staining results, follow these guidelines:

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

Continued on next page

Silver Staining, Continued

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.

SilverQuest™ Microwave Silver Staining Protocol

The microwave protocol for silver staining NuPAGE® Gels with SilverQuest™ Silver Staining Kit is provided below. For the Basic Protocol and more details on the staining procedure, refer to the SilverQuest™ Silver Staining Kit Manual (IM-6070). This manual is available on our Web site at www.invitrogen.com or contact Technical Service (see page 51).

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

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

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.

Continued on next page

SilverQuest™ Microwave Silver Staining Protocol, continued

4. Add 100 ml of Sensitizing solution to the washed gel. Microwave at high power for 30 seconds. Remove the gel from the microwave and place it on a rotary shaker for 2 minutes at room temperature. Decant the Sensitizing solution.
5. Wash the gel twice in 100 ml ultrapure water. Microwave at high power for 30 seconds. At each wash step, remove the gel from the microwave and gently agitate it for 2 minutes at room temperature.
6. Place the gel in 100 ml of Staining solution. Microwave at high power for 30 seconds. Remove the gel from the microwave and gently agitate it for 5 minutes at room temperature.
7. Decant the Staining solution and wash the gel with 100 ml of ultrapure water for 20-60 seconds. Do not wash the gel for more than a minute.
8. Place the gel in 100 ml of Developing solution and incubate for 5 minutes at room temperature with gentle agitation on a rotary shaker. **Do not microwave**.
9. Once the desired band intensity is achieved, immediately add 10 ml of Stopper directly to the gel still immersed in Developing solution and gently agitate the gel for 10 minutes. The color changes from pink to clear indicating that the end of development.
10. Wash the gel with 100 ml of ultrapure water for 10 minutes. For gel drying, see page 28.

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

Preparing Solutions for SilverXpress® Silver Staining

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

- Fixing solution

Methanol	100 ml
Acetic Acid	20 ml
Ultrapure water	90 ml

- Sensitizing solution

Methanol	100 ml
Sensitizer	5 ml
Ultrapure water	105 ml

- Staining solution

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

- Developing Solution

Developer	5 ml
Ultrapure water	95 ml

Continued on next page

Silver Staining, Continued

SilverXpress® Silver Staining Protocol

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

Note: Gels may be stored in the second Sensitizing Solution overnight, if desired. For gel drying, see page 28.

Step	Solution	Vol/Gel	NuPAGE® Gel Type	
			Tris –Acetate Gel	Bis-Tris Gel
1	Fix the gel in Fixing Solution.	200 ml	10 minutes	10 minutes
2A	Decant the Fixing Solution and incubate the gel in two changes of Sensitizing Solution.	100 ml	10 minutes	30 minutes
2B		100 ml	10 minutes	30 minutes
3A	Decant the Sensitizing Solution and rinse the gel twice with ultrapure water.	200 ml	5 minutes	10 minutes
3B		200 ml	5 minutes	10 minutes
4	Incubate the gel in Staining Solution.	100 ml	15 minutes	15 minutes
5A	Decant the Staining Solution and rinse the gel twice with ultrapure water.	200 ml	5 minutes	5 minutes
5B		200 ml	5 minutes	5 minutes
6	Incubate the gel in Developing Solution.	100 ml	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
8A	Decant the Stopping Solution and wash the gel three times in ultrapure water.	200 ml	10 minutes	10 minutes
8B		200 ml	10 minutes	10 minutes
8C		200 ml	10 minutes	10 minutes

Molecular Weight Calibration

Guidelines and apparent molecular weight values for the Novex® protein molecular weight standards in the NuPAGE® buffer system is provided on pages 39-41.

Coomassie® Staining

Introduction

Instructions are provided below for Coomassie® staining of NuPAGE® Gels using the SimplyBlue™ SafeStain, Colloidal Blue Staining Kit, and Coomassie® R-250 (see page 45 for ordering information).

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

Note

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

Materials Supplied by the User

You will need the following items for staining:

- Staining container
- Ultrapure water or deionized water
- Orbital Shaker
- Protein molecular weight standards (see page 45 for ordering information)
- Microwave oven and 20% NaCl (if using SimplyBlue™ SafeStain microwave protocol, see page 25)
- Methanol and acetic acid (if using Colloidal Blue Staining Kit, see page 26)

For Coomassie® R-250 staining

- 0.1% Coomassie® R-250 in 40% ethanol and 10% acetic acid
- Destaining Solution (10% ethanol and 7.5% acetic acid)

Continued on next page

SimplyBlue™ SafeStain Microwave Protocol

The microwave protocol for staining NuPAGE® Gels with SimplyBlue™ SafeStain is provided below. For the Basic Protocol and more details on the staining procedure, refer to the SimplyBlue™ SafeStain Manual (IM-6050). This manual is available on our Web site at www.invitrogen.com or contact Technical Service (see page 51).

The procedure is written for 1.0 mm mini-gels. For 1.5 mm mini-gels, use the values in parentheses.

Caution: Use caution while using the stain in a microwave oven. Do not overheat the staining solutions.

1. After electrophoresis, place the gel in 100 ml of ultrapure water in a loosely covered container and microwave on High (950 to 1100 watts) for 1 minute until the solution almost boils.
2. Gently shake the gel on an orbital shaker or rocker for 1 minute (2 minutes). Discard the water.
3. Repeat Steps 1 and 2 two more times.
4. Add 20 ml (30) ml of SimplyBlue™ SafeStain and microwave on High for 45 seconds to 1 minute (1.5 minutes) until the solution almost boils.
5. Shake the gel on an orbital shaker or rocker for 5 minutes (10 minutes). Detection limit: 20 ng BSA.
6. Wash the gel in 100 ml of ultrapure water for 10 minutes on a shaker. Detection limit: 10 ng BSA.
7. Add 20 ml of 20% NaCl for at least 5 minutes. Detection limit: 5 ng BSA. Gel can be kept for several weeks in the salt solution.
8. For gel-drying, see page 28.

Continued on next page

Colloidal Blue Staining Kit Protocol

A brief staining protocol for staining NuPAGE® Gels with the Colloidal Blue Staining Kit (see page 45 for ordering information) is provided below. For more details on the staining procedure, refer to the Manual (IM-6025). This manual is available on our Web site at www.invitrogen.com or contact Technical Service (see page 51).

Colloidal Blue Staining Kit Protocol for NuPAGE® Novex Tris-Acetate Gels

1. Prepare staining solution for a single gel consisting of 55 ml deionized water, 20 ml methanol, 5 ml Stainer B and 20 ml Stainer A. Be sure to shake Stainer B prior to making the solution.

Note: For two gels, double the volume of reagents used for staining.

2. Incubate the gel in this staining solution for a minimum of 3 hours and a maximum of 12 hours at room temperature with gentle shaking.
3. 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.
4. For gel-drying, see page 28.

Colloidal Blue Staining Kit Protocol for NuPAGE® Novex Bis-Tris Gels

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.

1. Prepare fixing solution consisting of 50% methanol, 10% acetic acid, and 40% deionized water.
2. Incubate the gel in the fixing solution for 10 minutes at room temperature with gentle shaking.
3. Prepare the staining solution containing 55 ml deionized water, 20 ml methanol, and 20 ml Stainer for one gel.
4. Incubate the gel in this staining solution for 10 minutes at room temperature with gentle shaking.
5. Add 5 ml Stainer B per gel to the staining solution from previous step. Continue staining for a minimum of 3 hours and a maximum of 16 hours.
6. Decant the staining solution and add 200 ml of deionized water per gel to the staining container. Gently shake the gel in water for at least 7 hours. Gel will have a clear background after 7 hours in water.
7. For gel-drying, see page 28.

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

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

Coomassie® Staining, Continued

Coomassie® R-250 Microwave Staining Protocol

The coomassie® staining protocol described below is recommended for staining NuPAGE® 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 of staining solution prepared in Step 1.
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 100 ml of destain solution prepared in Step 6.
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. **Note:** The NuPAGE® Gels destain faster than other Novex® Gels. To prevent over destaining of NuPAGE® Gels if destaining overnight, dilute the destain solution by adding 100 ml of deionized water to 100 ml of the destain solution in the staining container.
10. For-gel-drying, see page 28.

Molecular Weight Calibration

Guidelines and apparent molecular weight values for the Novex® protein molecular weight standards in the NuPAGE® buffer system is provided on pages 39-41.

Gel Drying

Introduction

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

We recommend drying Novex® Pre-Cast gels using passive air-drying methods such as DryEase® Mini-Gel Drying System (see page 45 for ordering information). If certain applications require drying the gel using vacuum drying, follow the recommendations on page 30 to minimize cracking of vacuum dried gels.

Note

Do not leave colloidal Coomassie® stained gels in Gel-Dry™ solution (or any equilibration solution which contains > 20% alcohol) for more than 5 minutes. Gels left in this solution for longer than 5 minutes will lose band intensity and detection limits may increase.

Materials Supplied by the User

You will need the following items. Ordering information is provided on page 45.

- 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

DryEase® Mini-Gel Drying System

A brief gel drying protocol using the DryEase® Mini-Gel Drying System is provided below. For more details on this system, refer to the DryEase® Mini-Gel Drying System manual (IM-2380). This manual is available for downloading from our Web site at www.invitrogen.com or contact Technical Service (see page 51).

Wear gloves while handling gels and gel drying solution.

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 coomassie® stained gels 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 sheets of cellophane per gel from the package.
6. Immerse one sheet of cellophane in the Gel-Dry™ Drying Solution. Allow 15-20 seconds for complete wetting before adding additional sheets. Do not soak the cellophane sheets for more than 2 minutes.

Continued on next page

DryEase® Mini-Gel Drying System, continued

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

Continued on next page

Vacuum Drying

General guidelines are provided below to minimize cracking during vacuum drying of gels. For detailed instructions, 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 19. 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 solution does not interfere with autoradiography.

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

Note: Do not incubate coomassie® stained gels 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 remove any air bubbles.

Use Proper Gel Dryer Set-up:

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

Ensure Gel is Completely Dry:

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

- Check the temperature of the gel

  The temperature of the dried should be the same as the temperature of the surrounding gel drying surface. If the temperature of the dried gel is cooler, then the gel is not completely dried.
- Check for moisture in the tubing connecting the gel dryer to the vacuum pump

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

Western Blotting

Introduction

Instructions are provided below for blotting NuPAGE® Gels using the XCell II™ Blot Module (see page 45 for ordering information). For more information on the XCell II™ Blot Module, refer to the manual (IM-9051). This manual is available on our Web site at www.invitrogen.com or contact Technical Service (see page 51).

See page 36 for Semi-Dry Blotting.

NuPAGE® Antioxidant

The NuPAGE® Antioxidant is used in the transfer buffer for blotting reduced proteins and prevents the proteins from reoxidizing and maintains the proteins in a reduced state (see page 14). Proteins are oxidized during blotting at a slower rate in the neutral pH environment of NuPAGE® blotting than in a higher pH blotting system. The major cause of reoxidation during blotting is the oxidizing effect of the anode electrochemistry.

NuPAGE® Transfer Buffer

We recommend using the NuPAGE® Transfer Buffer (see page 45 for ordering information) for western transfer of NuPAGE® Gels as the transfer buffer maintains the neutral pH environment established during NuPAGE® electrophoresis.

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

Materials Supplied by the User

- Blotting membranes (see page 45 for ordering information)
- Filter paper
- Methanol (if using PVDF membranes)
- XCell II™ Blot module
- NuPAGE® Transfer Buffer
- NuPAGE® Antioxidant for reduced samples
- MagicMark™ Western Protein Standard (see page 45 for ordering information)
- Deionized water

Continued on next page

Preparing NuPAGE® Transfer Buffer

Prepare 1000 ml of 1X NuPAGE® Transfer Buffer using the NuPAGE® Transfer Buffer (20X) as follows:

	Reduced Samples	Non-Reduced Samples
NuPAGE® Transfer Buffer (20X)	50 ml	50 ml
NuPAGE® Antioxidant	1 ml	--
Methanol	100 ml*	100 ml*
Deionized Water	849 ml	850 ml
Total Volume	1000 ml	1000 ml

*NuPAGE® Transfer Buffer with 10% methanol provides optimal transfer of a single gel in the blot module. If you are transferring two gels in the blot module, increase the methanol content to 20% to ensure efficient transfer of both gels.

See page 47 for a recipe of the NuPAGE® Transfer Buffer, if you are preparing your own transfer buffer.

Preparing Blotting Pads

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

Preparing Transfer Membrane and Filter

Cut selected transfer membrane and filter paper to the dimensions of the gel or use Novex® pre-cut membrane/filter paper sandwiches (see page 45 for ordering information.

- **PVDF membrane**—Pre-wet PVDF membrane for 30 seconds in methanol, ethanol, or isopropanol. Briefly rinse in deionized water, then place in a shallow dish with 50 ml of 1X NuPAGE® Transfer Buffer for several minutes.
- **Nitrocellulose**—Place the membrane directly into a shallow dish containing 50 ml of 1X NuPAGE® Transfer Buffer for several minutes.
- **Filter paper**—Soak the filter paper briefly in 1X NuPAGE® 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 19, remove wells with the Gel Knife.
2. Place a piece of pre-soaked filter paper on top of the gel, and lay just above the slot in the bottom of the cassette, leaving the "foot" of the gel uncovered. Keep the filter paper saturated with the transfer buffer and remove all trapped air bubbles by gently rolling over the surface using a glass pipette as a roller.
3. Turn the plate over so the gel and filter paper are facing downwards over a gloved hand or clean flat surface.
4. Use the Gel Knife to push the foot out of the slot in the plate and the gel will fall off.
5. When the gel is on a flat surface, cut the "foot" off the gel with the gel knife.
6. Wet the surface of the gel with transfer buffer and position the pre-soaked transfer membrane on the gel, ensuring all air bubbles have been removed.
7. Place another pre-soaked anode filter paper on top of the membrane. Remove any trapped air bubbles.
8. Place two soaked blotting pads into the cathode (-) core of the blot module. The cathode core is the deeper of the two cores and the corresponding electrode plate is a darker shade of gray. Carefully pick up the gel membrane assembly and place on blotting pad in the same sequence, such that the gel is closest to the cathode core (see Figure 1, next page).
9. Add enough pre-soaked blotting pads to rise to 0.5 cm over rim of cathode core. Place the anode (+) core on top of the pads. The gel/membrane assembly should be held securely between the two halves of the blot module ensuring complete contact of all components.
10. Position the gel/membrane assembly and blotting pads in the cathode core of the XCell II™ Blot Module to fit horizontally across the bottom of the unit. There should be a gap of approximately 1 cm at the top of the electrodes when the pads and assembly are in place.
11. Hold the blot module together firmly and slide it into the guide rails on the lower buffer chamber. The blot module will only fit into the unit one way, so the (+) sign can be seen in the upper left hand corner of the blot module. Properly placed, the inverted gold post on the right hand side of the blot module will fit into the hole next to the upright gold post on the right side of the lower buffer chamber.
12. Place the Gel Tension Wedge so that its vertical face is against the blot module. Lock the Gel Tension Wedge by pulling the lever forward.

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Western Transfer Using the XCell II™ Blot Module, continued

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

Transferring Two Gels in One Blot Module

1. Prepare 1X NuPAGE® Transfer Buffer containing 20% methanol as described on page 32.
2. Repeat Steps 1–7 above twice to make two gel/membrane sandwiches.
3. Place two pre-soaked pads on cathode shell of blot module. Place first gel/membrane assembly on pads in correct orientation, so gel is closest to the cathode core. (See Figure 2).
4. Add another pre-soaked blotting pad on top of first membrane assembly.
5. Position second gel/membrane assembly on top of blotting pad in the correct orientation so the gel is closest to the cathode side.
6. Proceed with steps 8–13 from **Transferring One Gel**.
7. Refer to **Recommended Transfer Conditions** on the next page for transfer conditions.

Figure 1

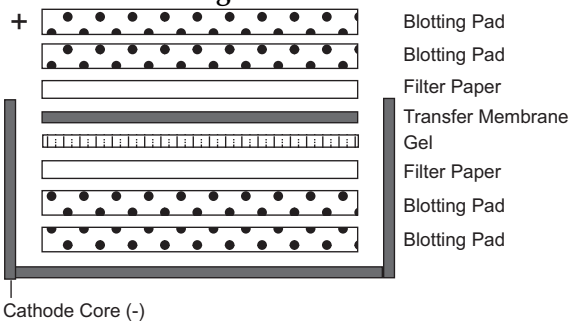
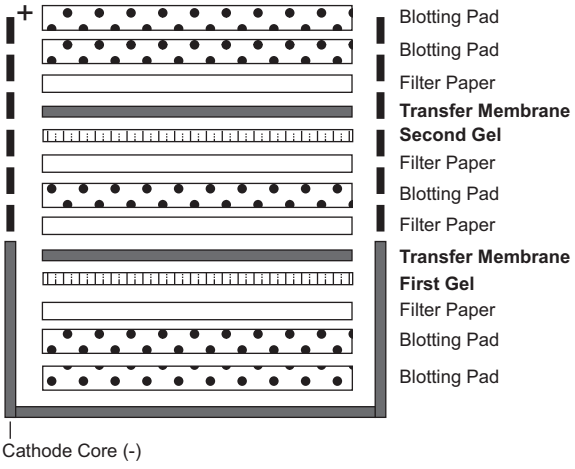


Figure 2



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Recommended Transfer Conditions

The transfer conditions for NuPAGE® Gels using the XCell II™ Blot Module are listed in the table below.

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

Gel	Transfer Buffer	Membrane	Power Conditions
NuPAGE® Novex Bis-Tris Gel	1X NuPAGE® Transfer Buffer with 10% methanol* 0.1% NuPAGE® Antioxidant for reduced samples	Nitrocellulose or PVDF	30 Volts constant for 1 hour Expected Current Start: 170 mA End: 110 mA
NuPAGE® Novex Tris-Acetate Gel	1X NuPAGE® Transfer Buffer with 10% methanol* 0.1% NuPAGE® Antioxidant for reduced samples	Nitrocellulose or PVDF	30 Volts constant for 1 hour Expected Current Start: 220 mA End: 180 mA

*NuPAGE® Transfer Buffer with 10% methanol provides optimal transfer conditions when blotting a single gel in a blot unit. If transferring two gels within a blot unit, increase the methanol content to 20% to ensure even and efficient transfer of both gels.

Alternate Transfer Buffers

The NuPAGE® Transfer buffer (with NuPAGE Antioxidant, if samples are reduced) is the optimal buffer for western transfer of NuPAGE® gels. However, you can use the Tris-Glycine Transfer Buffer (1X) or TBE Transfer Buffer (1/2X) for blotting NuPAGE® gels. The NuPAGE® Antioxidant is less functional if added to the Tris-Glycine and TBE buffers.

Carbonate and CAPS transfer buffers are not recommended for blotting of NuPAGE® Novex Pre-Cast Gels. The NuPAGE® Antioxidant is ineffective at pH> 9 and will not work when used with the Carbonate or CAPS transfer buffers.

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Semi-Dry Blotting of NuPAGE® Novex Bis-Tris Gels

The NuPAGE® Novex Bis-Tris Gels do not transfer efficiently using a semi-dry transfer cell as compared to blotting with XCell II™ Blot Module. If you decide to use semi-dry blotting for NuPAGE® Novex Bis-Tris Gels, use the protocol provided below to ensure efficient transfer of proteins.

1. Prepare 100 ml of 2X NuPAGE® Transfer Buffer from 20X NuPAGE® Transfer Buffer as follows:

NuPAGE® Transfer Buffer (20X)	10.0 ml
NuPAGE® Antioxidant (for reduced sample)	0.1 ml
Methanol	10.0 ml
Deionized Water	79.9 ml
Total Volume	100 ml

   If you are blotting large proteins, see the **Note** below.
2. Soak the filter paper and transfer membrane in the transfer buffer.
   - If you are using Novex® pre-cut membrane/filter sandwiches, use three filter papers (0.4 mm/filter in thickness) on each side of the gel or membrane.
   - If you are not using the Novex® pre-cut membrane/filter sandwiches, use two thick filter papers.
3. Assemble the gel/membrane/filter paper sandwich on top of the anode plate as follows:

   Filter Paper
   Filter Paper
   Filter Paper
   Membrane
   Gel
   Filter Paper
   Filter Paper
   Filter Paper
4. Perform the transfer at 15 V constant for 15 minutes if you are using the Bio-Rad Trans-Blot Semi-Dry Transfer Cell. For any other semi-dry transfer cell, follow the manufacturer's recommendations.

   Note: For transfer of large proteins (>100 kDa), pre-equilibrate the gel in 2X NuPAGE® Transfer Buffer (without methanol) containing 0.02-0.04% SDS for 10 minutes before assembling the sandwich.

Using ZOOM® Gels

ZOOM® Gels

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

Two types of ZOOM® Gels are available (see page 45 for ordering information)

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

Second Dimension Electrophoresis

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

Before Starting

You will need the following items:

- 4X NuPAGE® LDS Sample Buffer (see page 45 for ordering information)
- NuPAGE® Sample Reducing Agent (see page 45 for ordering information)
- NuPAGE® Novex 4-12% Bis-Tris ZOOM® Gel or Novex® 4-20% Tris-Glycine ZOOM™ Gel (see page 45 for ordering information)
- Appropriate running buffer depending on the type of gel you are using (see page 45 for ordering information)
- 0.5% agarose solution
- Iodoacetamide
- Plastic flexible ruler or thin weighing spatula
- 15 ml conical tubes
- Water bath set at 55°C or 65°C
- XCell *SureLock*™ Mini-Cell (see page 45 for ordering information)
- Protein molecular weight marker (see page 45 for ordering information)

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 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 fresh by adding 116 mg of 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 to **SDS-PAGE**, next page. Use the equilibrated IPG strip immediately for second dimension.

Continued on next page

SDS-PAGE

A protocol for SDS-PAGE is provided below using ZOOM® Gels with the XCell *SureLock*™ Mini-Cell. You may download the XCell *SureLock*™ Mini-Cell manual from our web site at www.invitrogen.com or contact Technical Service (see page 51). If you are using any other electrophoresis system, refer to the manufacturer's recommendations.

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

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

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, and
- Secondary structure of the protein

The same 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 in the NuPAGE® 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 (collagens, histones, or highly hydrophobic membrane proteins) and in peptides, where the effect of local secondary structure becomes magnified relative to the total size of the peptide.

Buffer Systems

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

Continued on next page

Calibrating Protein Molecular Weight, Continued

Assigned Apparent Molecular Weights

The apparent molecular weight values currently provided with the Novex® molecular weight standards were derived from the construction of a calibration curve in the Tris-Glycine SDS-PAGE System. We have now calculated and assigned apparent molecular weights for the Novex® protein standards in several buffer systems including the NuPAGE® buffer system. 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 the NuPAGE® Buffer System. You may generate calibration curves in your lab with any other manufacturer's standards.

Mark 12™ Unstained Standard	NuPAGE® (4-12%) Bis-Tris/MES	NuPAGE® (4-12%) Bis-Tris/MOPS	NuPAGE® (3-8%) Tris-Acetate
Myosin	200 kDa	200 kDa	200 kDa
β Galactosidase	116.3 kDa	116.3 kDa	116.3 kDa
Phosphorylase B	97.4 kDa	97.4 kDa	97.4 kDa
Bovine Serum Albumin	66.3 kDa	66.3 kDa	66.3 kDa
Glutamic Dehydrogenase	55.4 kDa	55.4 kDa	55.4 kDa
Lactate Dehydrogenase	36.5 kDa	36.5 kDa	36.5 kDa
Carbonic Anhydrase	31 kDa	31 kDa	31 kDa
Trypsin Inhibitor	21.5 kDa	21.5 kDa	N/A
Lysozyme	14.4 kDa	14.4 kDa	N/A
Aprotinin	6 kDa	6 kDa	N/A
Insulin B Chain	3.5 kDa	N/A	N/A
Insulin A Chain	2.5 kDa	N/A	N/A

MultiMark® Multi-Colored Standard	NuPAGE® (4-12%) Bis-Tris/MES	NuPAGE® (4-12%) Bis-Tris/MOPS	NuPAGE® (3-8%) Tris-Acetate
Myosin	185 kDa	188 kDa	209 kDa
Phosphorylase B	98 kDa	97 kDa	111 kDa
Glutamic Dehydrogenase	52 kDa	52 kDa	52 kDa
Carbonic Anhydrase	31 kDa	33 kDa	34 kDa
Myoglobin—Blue	19 kDa	21 kDa	N/A
Myoglobin—Red	17 kDa	19 kDa	N/A
Lysozyme	11 kDa	12 kDa	N/A
Aprotinin	6 kDa	N/A	N/A
Insulin	3 kDa	N/A	N/A

Continued on next page

Calibrating Protein Molecular Weight, Continued

Assigned Apparent Molecular Weights, continued

SeeBlue® Pre-Stained Standard	NuPAGE® (4-12%) Bis-Tris/MES	NuPAGE® (4-12%) Bis-Tris/MOPS	NuPAGE® (3-8%) Tris-Acetate
Myosin	188 kDa	191 kDa	210 kDa
BSA	62 kDa	64 kDa	71 kDa
Glutamic Dehydrogenase	49 kDa	51 kDa	55 kDa
Alcohol Dehydrogenase	38 kDa	39 kDa	41 kDa
Carbonic Anhydrase	28 kDa	28 kDa	N/A
Myoglobin	18 kDa	19 kDa	N/A
Lysozyme	14 kDa	14 kDa	N/A
Aprotinin	6 kDa	N/A	N/A
Insulin	3 kDa	N/A	N/A

SeeBlue® Plus2 Pre-Stained Standard	NuPAGE® (4-12%) Bis-Tris/MES	NuPAGE® (4-12%) Bis-Tris/MOPS	NuPAGE® (3-8%) Tris-Acetate
Myosin	188 kDa	191 kDa	210 kDa
Phosphorylase B	98 kDa	97 kDa	111 kDa
BSA	62 kDa	64 kDa	71 kDa
Glutamic Dehydrogenase	49 kDa	51 kDa	55 kDa
Alcohol Dehydrogenase	38 kDa	39 kDa	41 kDa
Carbonic Anhydrase	28 kDa	28 kDa	N/A
Myoglobin	17 kDa	19 kDa	N/A
Lysozyme	14 kDa	14 kDa	N/A
Aprotinin	6 kDa	N/A	N/A
Insulin	3 kDa	N/A	N/A

Troubleshooting

Introduction

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

Problem	Cause	Solution
Run taking longer time	Running buffer too dilute	Make fresh running buffer as described on page 14 and do not adjust 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.
Streaking of proteins	• Sample overload • High salt concentration in the sample • Sample precipitates • Contaminants such as membranes or DNA complexes in the sample	• Load the appropriate amount of protein as described on page 6. • Decrease the salt concentration of your sample using dialysis or gel filtration. • Increase the concentration of SDS in your sample, if necessary to maintain the solubility of the protein. • Centrifuge or clarify your sample to remove particulate contaminants.
Dumbbell shaped bands after electrophoresis	Loading a large volume of sample causes incomplete stacking of the entire sample. This effect is more intensified for larger proteins	Load the appropriate volume of sample per well as described on page 6. If your sample is too dilute, concentrate the sample using ultrafiltration.

Continued on next page

Using incorrect Buffers with NuPAGE® Bis-Tris Gels

See the table below for the outcome of your results if you accidentally used an incorrect buffer instead of the NuPAGE® MOPS/MES SDS Running Buffer and NuPAGE® LDS Sample Buffer on the NuPAGE® Bis-Tris Gels.

If you used the…	Instead of the….	Then….
NuPAGE® MES SDS Running Buffer	NuPAGE® MOPS SDS Running Buffer	the run time of the gel is decreased by ~10-15 minutes.
		there is decreased separation and resolution for proteins > 36 kDa.
NuPAGE® MOPS SDS Running Buffer	NuPAGE® MES SDS Running Buffer	the run time of the gel is increased by ~10-15 minutes.
		the lower molecular weight proteins (<14 kDa), which are normally well resolved, are not resolved while the high molecular weight proteins are resolved more than normal.
Novex® Tris-Glycine SDS Sample Buffer	NuPAGE® LDS Sample Buffer	some bands are not very sharp and there is increased protein fragmentation.
Novex® Tricine SDS Sample Buffer	NuPAGE® LDS Sample Buffer	the band sharpness is not affected, but the lanes will be slightly wider due to the increased amount of SDS and buffer salts from the Tricine Sample Buffer.
Novex® Tris-Glycine SDS Running Buffer and the Novex® Tris-Glycine SDS Sample Buffer	NuPAGE® MOPS or MES SDS Running Buffer and the NuPAGE® LDS Sample Buffer	the gel will have an extremely long run time of 3-4 hours due to the low migration of the glycine ions at neutral pH.
		the sensitivity of the staining for high molecular weight proteins is decreased.
		the bands are more compressed at the bottom of the gel, regardless of the gel percentage and the bands have a cupped appearance at the bottom of the band.
Novex® Tricine SDS Running Buffer and the Tricine SDS Sample Buffer	NuPAGE® MOPS or MES SDS Running Buffer and the NuPAGE® LDS Sample Buffer	the run time of the gel is increased by 1-2 hours due to the slow migration of the tricine ions at neutral pH.
		there may be background streaking in the lanes.

Continued on next page

Using incorrect Buffers with NuPAGE® Tris-Acetate Gels

Refer to the table below for the outcome of your results if you accidentally used a incorrect buffer system instead of the NuPAGE® Tris-Acetate SDS Running Buffer and NuPAGE® LDS Sample Buffer on the NuPAGE® Tris-Acetate Gels.

Sample Buffer	Running Buffer	Antioxidant	Results
Novex® Tris-Glycine SDS	NuPAGE® Tris-Acetate SDS	Yes	Fuzzy, smeary bands.
Novex® Tricine SDS	NuPAGE® Tris-Acetate SDS	Yes	Bands are not very sharp.
NuPAGE® LDS	NuPAGE® MES SDS or NuPAGE® MOPS SDS	Yes	Bands are diffuse and have a "U" shape. More low molecular weight proteins are visible.
NuPAGE® LDS	Novex® Tris-Glycine SDS	No	The run time is twice as long as the Tris-Acetate Buffer system. The band resolution is poor.
NuPAGE® LDS	Novex® Tricine SDS	No	The run time is 10-15 minutes faster than the Tris-Acetate Buffer system. Reduced protein bands are diffuse while non-reduced large molecular weight protein bands are smeary.
Novex® Tris-Glycine SDS	Novex® Tris-Glycine SDS	No	The run time is much longer than the Tris-Acetate Buffer system and the bands are very faint with a streaking background. Fewer low molecular weight bands are resolved.
Novex® Tricine SDS	Novex® Tricine SDS	No	The run time is 10-15 minutes faster than the Tris-Acetate Buffer system and reduced protein bands are not very sharp. The overall performance is acceptable.

Accessory Products

Electrophoresis Reagents

A large variety of electrophoresis reagents and apparatus are available from Invitrogen for the separation and analysis of proteins. Ordering information is provided below. For more information, visit our Web site at www.invitrogen.com or call Technical Service (see page 51).

Product	Application	Quantity	Catalog no.
XCell *SureLock*™ Mini-Cell	For convenient, leak-free vertical electrophoresis	1 unit	EI0001
XCell II™ Blot Module	For blotting of mini-gels	1 unit	EI9051
PowerEase® 500 Power Supply	Programmable power supply	1 unit	EI8600
DryEase® Mini-Gel Drying System	Even, crack-free drying of gels using passive evaporation	1 Kit	NI2387
StainEase® Staining Tray	For even staining and destaining of gels	2/pack	NI2400
Gel-Dry™ Drying Solution	For even drying of gels	500 ml	LC4025
NuPAGE® LDS Sample Buffer (4X)	For preparing protein samples for denaturing gel electrophoresis	10 ml 250 ml	NP0007 NP0008
NuPAGE® MOPS SDS Running Buffer (20X)	Denaturing running buffer for NuPAGE® Bis-Tris Gels	500 ml	NP0001
NuPAGE® MES SDS Running Buffer (20X)	Denaturing running buffer for NuPAGE® Bis-Tris Gels	500 ml	NP0002
NuPAGE® Tris-Acetate SDS Running Buffer (20X)	Denaturing running buffer for NuPAGE® Tris-Acetate Gels	500 ml	LA0041
NuPAGE® Antioxidant	For maintaining reducing conditions during electrophoresis and blotting of the NuPAGE® Gels	15 ml	NP0005
NuPAGE® Sample Reducing Agent (10X)	For preparing reduced protein samples for NuPAGE® Buffers	250 µl 10 ml	NP0004 NP0009
NuPAGE® Transfer Buffer (20X)	For Western transfer of NuPAGE® Gels	125 ml	NP0006
Novex® Tris-Glycine Native Running Buffer (10X)	Native running buffer for NuPAGE® Novex Tris-Acetate Gels	500 ml	LC2672
Novex® Tris-Glycine Native Sample Buffer (2X)	For preparing protein samples for native gel electrophoresis	20 ml	LC2673
Nitrocellulose Membrane 0.2 µm	Immunoblotting and amino acid analysis	20 blots	LC2000
Invitrolon™ PVDF membranes 0.45 µm	Immunoblotting, protein sequencing, and amino acid analysis	20 blots	LC2005
PVDF membranes 0.2 µm			LC2002

Continued on next page

Protein Standards and Stains

Ordering information for stains and protein molecular weights is provided below. For more information, visit our Web site at www.invitrogen.com or contact Technical Service (see page 51).

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 protein molecular weight	1 ml	LC5677
MagicMark™ Western Standard	For protein molecular weight estimation on western blots	250 µl	LC5600
SeeBlue® Pre-Stained Standard	For monitoring the progress of your run and evaluating transfer efficiency	500 µl	LC5625
SeeBlue® Plus2 Pre-Stained Standard	For visualizing protein molecular weight range and evaluating transfer efficiency	500 µl	LC5925
MultiMark® Multi-Colored Standard	For visualizing protein molecular weight range and evaluating transfer efficiency	500 µl	LC5725
BenchMark® Protein Ladder	For estimating the apparent protein molecular weight	2 x 250 µl	10747-012

Recipes

NuPAGE® MOPS SDS Running Buffer

The NuPAGE® MOPS SDS Running Buffer (20X) is available from Invitrogen (see page 45)

50 mM MOPS
50 mM Tris base
0.1% SDS
1 mM EDTA
pH 7.7

1. To prepare 500 ml of 20 X NuPAGE® MOPS SDS Running Buffer, dissolve the following reagents to 400 ml ultrapure water:

MOPS	104.6 g
Tris Base	60.6 g
SDS	10 g
EDTA	3.0 g

2. Mix well and adjust the volume to 500 ml with ultrapure water.
3. Store at +4°C. The buffer is stable for 6 months when stored at +4°C.
4. For electrophoresis, dilute this buffer to 1X with water (see page 15). The pH of the 1X solution is 7.7. Do not use acid or base to adjust the pH.

NuPAGE® MES SDS Running Buffer

The NuPAGE® MES SDS Running Buffer (20X) is available from Invitrogen (see page 45)

50 mM MES
50 mM Tris base
0.1% SDS
1 mM EDTA
pH 7.3

1. To prepare 500 ml of 20 X NuPAGE® MES SDS Running Buffer, dissolve the following reagents to 400 ml ultrapure water:

MES	97.6 g
Tris Base	60.6 g
SDS	10 g
EDTA	3.0 g

2. Mix well and adjust the volume to 500 ml with ultrapure water.
3. Store at +4°C. The buffer is stable for 6 months when stored at +4°C.
4. For electrophoresis, dilute this buffer to 1X with water (see page 15). The pH of the 1X solution is 7.3. Do not use acid or base to adjust the pH.

Continued on next page

NuPAGE® Tris-Acetate SDS Running Buffer

The NuPAGE® Tris-Acetate SDS Running Buffer (20X) is available from Invitrogen (see page 45)

50 mM Tricine
50 mM Tris base
0.1% SDS
pH 8.24

1. To prepare 500 ml of 20 X NuPAGE® Tris-Acetate SDS Running Buffer, dissolve the following reagents to 400 ml ultrapure water:

Tricine	89.5 g
Tris Base	60.6 g
SDS	10 g

2. Mix well and adjust the volume to 500 ml with ultrapure water.
3. Store at +4°C. The buffer is stable for 6 months when stored at +4°C.
4. For electrophoresis, dilute this buffer to 1X with water (see page 15). The pH of the 1X solution is 8.24. Do not use acid or base to adjust the pH.

NuPAGE® Transfer Buffer

The NuPAGE® Transfer Buffer (20X) is available from Invitrogen (see page 45)

25 mM Bicine
25 mM Bis-Tris (free base)
1 mM EDTA
pH 7.2

1. To prepare 125 ml of 20 X NuPAGE® Transfer Buffer, dissolve the following reagents to 100 ml ultrapure water:

Bicine	10.2 g
Bis-Tris (free base)	13.1 g
EDTA	0.75 g

2. Mix well and adjust the volume to 125 ml with ultrapure water.
3. Store at +4°C. The buffer is stable for 6 months when stored at +4°C.
4. For western transfer, dilute this buffer to 1X with water (see page 32). The pH of the 1X solution is 7.2. Do not use acid or base to adjust the pH.

Continued on next page

NuPAGE® LDS Sample Buffer

The NuPAGE® LDS Sample Buffer (4X) is available from Invitrogen (see page 45)

106 mM Tris HCl
141 mM Tris base
2% LDS
10% Glycerol
0.51 mM EDTA
0.22 mM SERVA® Blue G250
0.175 mM Phenol Red
pH 8.5

1. To prepare 10 ml of 4 X NuPAGE® LDS Sample Buffer, dissolve the following reagents to 8 ml ultrapure water:

Tris HCl	0.666 g
Tris Base	0.682 g
LDS	0.800 g
EDTA	0.006 g
Glycerol	4 g
SERVA® Blue G250 (1% solution)	0.75 ml
Phenol Red (1% solution)	0.25 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.
4. For electrophoresis, prepare your samples in this buffer as described on page 11. The pH of the 1X solution is 8.5. Do not use acid or base to adjust the pH.

Tris-Glycine Native Sample Buffer

The Tris-Glycine Native Sample Buffer (2X) is available from Invitrogen (see page 45)

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

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

4 M Tris HCl	4 ml
10% Glycerol	2 ml
0.1% Bromophenol Blue	0.5 ml
Deionized Water	3.5 ml

2. Mix well and adjust the pH of the solution is 8.6.
3. Store at +4°C. The buffer is stable for 6 months when stored at +4°C.
4. Use this buffer to prepare samples for non-denaturing NuPAGE® Tris-Acetate gel electrophoresis (see page 11).

Continued on next page

Tris-Glycine Native Running Buffer

The Tris-Glycine Native Running Buffer (10X) is available from Invitrogen (see page 45)

25 mM Tris base
192 mM Glycine
pH 8.3

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

Tris Base	29 g
Glycine	144 g

2. Mix well and adjust the volume to 1000 ml with ultrapure water.
3. Store at room temperature. The buffer is stable for 6 months when stored at room temperature.
4. For native electrophoresis, dilute this buffer to 1X with water (see page 16). The pH of the 1X solution is 8.3. Do not use acid or base to adjust the pH.

Technical Service

World Wide Web

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

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

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

http://www.invitrogen.com

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

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Corporate Headquarters:
Invitrogen Corporation
1600 Faraday Avenue
Carlsbad, CA 92008 USA
Tel: 1 760 603 7200
Tel (Toll Free): 1 800 955 6288
Fax: 1 760 602 6500
E-mail:
tech_service@invitrogen.com

Japanese Headquarters:
Invitrogen Japan K.K.
Nihonbashi Hama-Cho Park Bldg. 4F
2-35-4, Hama-Cho, Nihonbashi
Tel: 81 3 3663 7972
Fax: 81 3 3663 8242
E-mail: jpinfo@invitrogen.com

European Headquarters:
Invitrogen Ltd
Inchinnan Business Park
3 Fountain Drive
Paisley PA4 9RF, UK
Tel: +44 (0) 141 814 6100
Tech Fax: +44 (0) 141 814 6117
E-mail: eurotech@invitrogen.com

MSDS Requests

To request an MSDS, visit our Web site at www.invitrogen.com. On the home page, go to 'Technical Resources', select 'MSDS', and follow instructions on the page.

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Limited Use Label License No. 71: NuPAGE® Bis-Tris Gel System

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United States Headquarters:
Invitrogen Corporation
1600 Faraday Avenue
Carlsbad, California 92008
Tel: 1 760 603 7200
Tel (Toll Free): 1 800 955 6288
Fax: 1 760 603 7229
Email: tech_service@invitrogen.com

European Headquarters:
Invitrogen Ltd
3 Fountain Drive
Inchinnan Business Park
Paisley PA4 9RF, UK
Tel (Free Phone Orders): 0800 269 210
Tel (General Enquiries): 0800 5345 5345
Fax: +44 (0) 141 814 6287
Email: eurotech@invitrogen.com

International Offices:
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