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Novex™ Midi Gel System
USER GUIDE

A system for electrophoresis, blotting, and of midi gels

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Product description

Introduction

The Novex™ Midi Gel System is a discontinuous SDS-PAGE, pre-cast polyacrylamide midi gel system designed to perform higher throughput electrophoresis.

Three types of Novex™ Midi Gel System are available for purchase:

- **The NuPAGE™ Midi Gel System** is a revolutionary neutral pH, discontinuous SDS-PAGE 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 page 7 for details on the NuPAGE™ Electrophoresis System.
- **The Tris-Glycine Midi Gel System** is based on the Laemmli System with minor modifications for maximum performance in the pre-cast format. The separating and stacking gels of Novex™ Tris-Glycine Midi Gels have a pH of 8.65, unlike traditional Laemmli gels that have a stacking gel pH of 6.8 and separating gel pH of 8.8. See page 8 for details on the Tris-Glycine Electrophoresis System.
- **The Tris-Glycine Plus Midi Gel System** is based on the Laemmli System and is modified to provide maximum performance in the pre-cast format with extended product shelf life.

Purpose of the manual

This manual provides the following information:

- An overview of the Novex™ Midi Gel System, NuPAGE™ Protein Electrophoresis System, Tris-Glycine Protein Electrophoresis System, and Tris-Glycine Plus Protein Electrophoresis System
- Preparing samples and running buffer
- Instructions for performing SDS-PAGE using the XCell4 *SureLock*™ Midi-Cell and Criterion™ Cell (Bio-Rad)
- Protocol for staining using the SimplyBlue™ Safestain and SYPRO™ Ruby Protein Stain
- Western blotting protocol using a Semi-Dry blotting apparatus or iBlot™ 2 Dry Blotting System
- Troubleshooting

Applications

The Novex™ Midi Gels are used to perform higher throughput electrophoresis:

- For separating proteins under denaturing conditions (NuPAGE™ Bis-Tris, NuPAGE™ Tris-Acetate, Novex™ Tris-Glycine, and Novex™ Tris-Glycine Plus Midi Gels)
- For separating proteins under non-denaturing (native) conditions (NuPAGE™ Tris-Acetate Novex™ Tris-Glycine, and Novex™ Tris-Glycine Plus Midi Gels)
- For protein sequencing using Edman sequencing (from gels or PVDF)

Note: Do not use the NuPAGE™ Bis-Tris Midi 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.

Types of gels

Novex™ Midi Gels are available in different acrylamide concentrations and well formats (see the following table). All gels are available in 1.0 mm thickness only.

Feature	Gel type			
	Bis-Tris	Tris-Acetate	Tris-Glycine	Tris-Glycine Plus
Separating Gel Acrylamide Concentration	8%, 10%, 4–12%	3–8%	8%, 10%, 12%, 4–12%, 4–20%, 8–16%	10%, 12%, 4–12%, 4–20%, 8–16%
Stacking Gel Acrylamide Concentration	4%	3.2%	4%	4%
Well Format	12+2, 20, and 26	12+2, 20, and 26	12+2, 20, and 26	12+2, 20, and 26

Note

Novex™ Midi Gels do not contain SDS. However, the midi gels are designed for performing denaturing gel electrophoresis.

Choosing a gel for your application

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

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

Size of the protein being separated

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

Amount of available material

The higher the number of wells, the lower the sample loading volume and vice versa (see page 6 for the recommended loading volumes for the various well formats). Based on the amount of your starting material available, you can choose from a variety of comb types.

Refer to the gel migration chart on our website at **thermofisher.com** to choose the right gel for your application. Choose a gel such that the molecules migrate about 70% of the length of gel for best resolution (shaded area on the gel migration chart).

Compatibility

The size of a Novex™ Midi Gel is 15 cm × 10.3 cm (gel size is 13 cm × 8.3 cm). We recommend using the XCell4 *SureLock*™ Midi-Cell (see page 30 for ordering information) for the electrophoresis of Novex™ Midi Gels to obtain optimal and consistent performance.

The Novex™ Midi Gels with Midi Gel Adapters are also compatible for use with the Criterion™ Cell available from Bio-Rad.

Downstream applications

- The Novex™ Midi Gels are compatible with most staining protocols including silver, Coomassie, and fluorescent stains.
- The Novex™ Midi Gels are suited for western transfer applications using a semi-dry or semi-wet transfer apparatus that can accommodate a midi gel, or the iBlot™ 2 Gel Transfer Device (page 22).

Kit contents and storage

Types of products

This manual is intended for use with the following products. For ordering information, visit **thermofisher.com** or contact Technical Support (page 32).

Product	Quantity
NuPAGE™ Novex™ Bis-Tris Midi Gels	Box of 10 gels
NuPAGE™ Novex™ Bis-Tris Midi Gels with Adapters	Box of 10 gels Box of 10 Midi Gel Adapters
NuPAGE™ Novex™ Tris-Acetate Midi Gels	Box of 10 gels
NuPAGE™ Novex™ Tris-Acetate Midi Gels with Adapters	Box of 10 gels Box of 10 Midi Gel Adapters
Novex™ Tris-Glycine Midi Gels	Box of 10 gels
Novex™ Tris-Glycine Midi Gels with Adapters	Box of 10 gels Box of 10 Midi Gel Adapters
Novex™ Tris-Glycine Plus Midi Gels	Box of 10 gels
Novex™ Tris-Glycine Plus Midi Gels with Adapters	Box of 10 gels Box of 10 Midi Gel Adapters

Shipping and storage

The table below describes the shipping and storage of Novex™ Midi Gels. **Do not freeze Novex™ Midi gels.**

Item	Shipping	Storage	Shelf life
NuPAGE™ Novex™ Bis-Tris Midi Gels	Room temperature	2°C to 8°C	12 months
NuPAGE™ Novex™ Tris-Acetate Midi Gels	Blue ice	2°C to 8°C	8 months
Novex™ Tris-Glycine Midi Gels	Blue ice	2°C to 8°C	4–8 weeks (depending on gel type)
Novex™ Tris-Glycine Plus Midi Gels	Blue ice	2°C to 8°C	6–12 months (depending on gel type)
Midi Gel Adapters	Room temperature	15°C to 30°C	Not applicable

Loading volumes

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

Well types	Recommended maximum load volume	Maximum protein load per band by detection method		
		Coomassie staining	Silver staining	Immunoblotting
12 + 2 Well 	45 µL: sample well 15 µL: marker well	0.7 µg/band	Scale your sample load for the sensitivity of your silver staining kit. A protein load of 1 ng/band is generally recommended.	Scale your sample load according to the sensitivity of your detection method.
20 Well 	25 µL	0.7 µg/band		
26 Well 	15 µL	0.4 µg/band		

NuPAGE™ electrophoresis system

System components

The NuPAGE™ Novex™ Midi Gel System consists of:

- NuPAGE™ Novex™ Bis-Tris [Bis (2-hydroxyethyl) imino-tris (hydroxymethyl) methane-HCl] Midi Gels for separating small to mid-size molecular weight proteins
- NuPAGE™ Novex™ Tris-Acetate Midi Gels for separating large molecular weight proteins
- NuPAGE™ LDS (lithium dodecyl sulfate) Sample Buffer
- NuPAGE™ Sample 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 Midi Gels
- NuPAGE™ Tris-Acetate SDS Running Buffer for NuPAGE™ Novex Tris-Acetate Midi Gels
- NuPAGE™ Transfer Buffer for blotting of NuPAGE™ Novex Midi Gels

NuPAGE™ Novex™ Midi Gels

The NuPAGE™ Novex™ Midi Gel is a 1.0 mm thick, wider (13 cm × 8.3 cm) format midi gel used for higher throughput electrophoresis of protein samples.

The NuPAGE™ Novex™ Midi Gels are used with the NuPAGE™ Bis-Tris or Tris-Acetate SDS Buffer System (see page 7) to produce a discontinuous SDS-PAGE system operating at neutral pH. The neutral pH environment during electrophoresis results in maximum stability of both proteins and gel matrix, providing better band resolution than other gel systems. See page 5 for types of gels available from Thermo Fisher Scientific.

NuPAGE™ Bis-Tris buffer system

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

- Chloride (Cl^-) 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 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.

NuPAGE™ LDS Sample Buffer

Use the NuPAGE™ LDS Sample Buffer (4X) to prepare samples for denaturing gel electrophoresis with the **NuPAGE**™ Novex Midi Gels.

The NuPAGE™ LDS Sample Buffer is formulated to reliably provide complete reduction of the disulfides under mild heating conditions (70°C for 10 minutes) and eliminate protein cleavage during sample preparation.

NuPAGE™ Sample Reducing Agent

The NuPAGE™ Sample Reducing Agent contains 500 mM dithiothreitol (DTT) at a 10X concentration and is available in a ready-to-use, stabilized liquid form (page 30). Use the NuPAGE™ Sample Reducing Agent to prepare samples for reducing gel electrophoresis with Novex™ Midi Gels.

NuPAGE™ Antioxidant

The reducing agents, DTT and β-mercaptoethanol, do not co-migrate through the gel with the sample in the neutral pH environment of NuPAGE™ Novex™ Midi Gels. Instead, the reducing agent tends to remain at the top of the gel and not migrate fully throughout the gel, resulting in the reoxidization of some proteins, producing 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 of NuPAGE™ gels under reducing conditions. The NuPAGE™ Antioxidant migrates with the proteins during electrophoresis of NuPAGE™ gels maintaining sample proteins that have been treated with reducing agents in a reduced state. The NuPAGE™ Antioxidant also protects sensitive amino acids such as methionine and tryptophan from oxidizing.

NuPAGE™ Running Buffers

Three NuPAGE™ Running Buffers are available for performing denaturing electrophoresis.

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

Note: 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.

NuPAGE™ Transfer Buffer

NuPAGE™ Transfer Buffer is recommended for western transfer of proteins from NuPAGE™ Novex Bis-Tris and Tris-Acetate Midi Gels. The buffer maintains the neutral pH environment established during gel electrophoresis, protects against modification of the amino acid side chains, and is compatible with N-terminal protein sequencing using Edman degradation.

Advantages

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

- Longer shelf life of up to 12 months due to improved gel stability
- Improved protein stability during electrophoresis at neutral pH resulting in sharper band resolution and accurate results
- Complete reduction of disulfides with absence of cleavage of Asp-Pro bonds under mild heating (70°C for 10 minutes) 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

Separation range

The NuPAGE™ Novex™ Midi Gels have a wider range of separation throughout the low and high molecular weight ranges.

By combining any of the NuPAGE™ Novex™ Bis-Tris Midi 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 Midi Gels resolve proteins in the molecular weight range of **36–400 kDa**.

To choose the correct NuPAGE™ Novex™ Midi Gel for your application, refer to the gel migration chart on our website at **thermofisher.com/novex**.

Tris-Glycine Midi Gel System

System components

The Novex™ Tris-Glycine Midi Gel System consists of:

- Novex™ Tris-Glycine Midi Gels for separating a wide range of proteins
- Novex™ Tris-Glycine SDS Sample Buffer for preparing samples using denaturing conditions
- Novex™ Tris-Glycine Native Sample Buffer for preparing samples using native conditions
- NuPAGE™ Sample Reducing Agent (see page 8 for details)
- Novex™ Tris-Glycine SDS Running Buffer for denaturing electrophoresis
- Novex™ Tris-Glycine Native Running Buffer for native electrophoresis
- Novex™ Tris-Glycine Transfer Buffer for blotting of Tris-Glycine Midi Gels

Novex™ Tris-Glycine Midi Gels

The Novex™ Tris-Glycine Midi Gel is a 1.0 mm thick, wider (13 cm × 8.3 cm) format midi gel used for higher throughput electrophoresis of protein samples.

The Novex™ Tris-Glycine Midi Gels are used with the Novex™ Tris-Glycine SDS Buffer System to produce a discontinuous Laemmli SDS-PAGE system. See page 5 for types of gels available from Thermo Fisher Scientific.

Tris-Glycine discontinuous buffer system

The Tris-Glycine discontinuous buffer systems involves three ions:

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

Novex™ Tris-Glycine SDS Sample Buffer

Use the Novex™ Tris-Glycine SDS Sample Buffer (2X) to prepare samples for denaturing gel electrophoresis with the Novex™ Tris-Glycine Midi Gels or Novex™ Tris-Glycine Plus Midi Gels.

The Novex™ Tris-Glycine SDS Sample Buffer formulation is based on the Laemmli formulation.

Novex™ Tris-Glycine Transfer Buffer

Use Tris-Glycine Transfer Buffer for western transfer of proteins from Tris-Glycine or Tris-Glycine Plus Midi Gels.

Separation range

The Novex™ Tris-Glycine Midi Gels have a wider range of separation throughout the low and high molecular weight ranges. The separating range of Tris-Glycine gels is **6–200 kDa**.

To choose the correct Novex™ Tris-Glycine Midi Gel for your application, refer to the gel migration chart on our website at **thermofisher.com/novex**.

Tris-Glycine Plus Midi Gel System

System components

The Novex™ Tris-Glycine Plus Midi Gel System consists of:

- Novex™ Tris-Glycine Plus Midi Gels for separating a wide range of proteins
- Novex™ Tris-Glycine SDS Sample Buffer for preparing samples using denaturing conditions (see page 9 for details)
- Novex™ Tris-Glycine Native Sample Buffer for preparing samples using native conditions
- NuPAGE™ Sample Reducing Agent (see page 8 for details)
- Novex™ Tris-Glycine SDS Running Buffer for denaturing electrophoresis
- Novex™ Tris-Glycine Native Running Buffer for native electrophoresis
- Novex™ Tris-Glycine Transfer Buffer for blotting of Tris-Glycine Plus Midi Gels (see page 9 for details)

Novex™ Tris-Glycine Plus Midi Gels

The Novex™ Tris-Glycine Plus Midi Gel is a 1.0 mm thick, wider (13 cm × 8.3 cm) format midi gel used for higher throughput electrophoresis of protein samples.

The Novex™ Tris-Glycine Plus Midi Gels are used with the Novex™ Tris-Glycine SDS Buffer System (see page 9) to produce a discontinuous Laemmli SDS-PAGE system. See page 5 for types of gels available from Thermo Fisher Scientific.

Tris-Glycine Plus discontinuous buffer system

The Tris-Glycine Plus Midi Gels use a proprietary buffer that is based on the Laemmli System and is modified to provide maximum performance in the pre-cast format with extended product shelf life.

Separation range

The separating range of Novex™ Tris-Glycine Plus Midi Gels is **6–200 kDa**.

To choose the correct Novex™ Tris-Glycine Midi Gel for your application, refer to the gel migration chart on our website at **thermofisher.com/novex**.

Guidelines for samples and markers

Reduced and non-reduced samples

For optimal results, it is **not recommended** to run reduced and non-reduced samples on the same gel.

If it is necessary 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.
- Do not add NuPAGE™ Antioxidant to the running buffer. The antioxidant will have a deleterious effect on the non-reduced samples. The bands will appear sharper on NuPAGE™ Midi Gels relative to other gel systems, even without the use of the antioxidant.

Protein molecular weight markers

The following protein molecular weight markers are recommended for use with the Novex™ Midi Gels (see page 30 for details).

- PageRuler™ Prestained Protein Ladder
- PageRuler™ Plus Prestained Protein Ladder
- NativeMark™ Unstained Protein Ladder
- HiMark™ Pre-stained and Unstained Protein Standard for determining molecular weight of large proteins
- MagicMark™ Western Protein Standard for Western blotting applications

The apparent molecular weight of the protein standards on a midi gel remains approximately the same as observed for a mini gel.

To obtain equivalent band intensities based on the recommended load for a 10-well mini gel, use the recommended protein standard load in the following table for each type of midi gel.

Recommended volume for 10-well mini gel	Recommended volume for midi gel		
	12 + 2 well	20 well	26 well
5 µL	7 µL + 2.5 µL	4 µL	2.5 µL
10 µL	14 µL + 5 µL	8 µL	5 µL

Prepare sample for denaturing electrophoresis

General guidelines

Denaturing electrophoresis can be performed using NuPAGE™ Bis-Tris, NuPAGE™ Tris-Acetate, Novex™ Tris-Glycine, and Novex™ Tris-Glycine Plus Midi Gels.

- Add the reducing agent to the sample within an hour before loading the gel. **For best results, add the reducing agent immediately prior to heating.**
- Avoid storing reduced samples for long periods. Even if frozen, samples will reoxidize during storage and produce inconsistent results.
- Do not use the NuPAGE™ Antioxidant as a sample reducing agent. The antioxidant is not efficient in reducing disulfide bonds. Use will result in partially reduced bands with substantial background smearing in the lane.

Materials needed

You will need the following items. See page 30 for ordering information.

- Protein sample and molecular weight marker
- Deionized water
- Appropriate Sample Buffer
  - NuPAGE™ LDS Sample Buffer (4X) for NuPAGE™ Midi Gels
  - Novex™ Tris-Glycine SDS Sample Buffer (2X) for Tris-Glycine Midi Gels or Novex™ Tris-Glycine Plus Midi Gels
- NuPAGE™ Sample Reducing Agent

Prepare samples for NuPAGE™ gels

1. Use the volumes in the following table to prepare samples in a total volume of 10 µL. To prepare samples in other volumes, scale the volume of reagents accordingly. See page 6 for the recommended protein load.

Reagent	NuPAGE™ Gel	
	Reduced	Non-Reduced
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 10 µL	to 10 µL

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

Prepare samples for Tris-Glycine or Tris-Glycine Plus gels

1. Use the volumes in the following table to prepare samples in a total volume of 10 µL. To prepare samples in other volumes, scale the volume of reagents accordingly. See page 6 for the recommended protein load.

Reagent	Tris-Glycine/ Tris-Glycine Plus Gel	
	Reduced	Non-Reduced
Sample	*x* µL	*x* µL
Tris-Glycine SDS Sample Buffer (2X)	5 µL	5 µL
NuPAGE™ Reducing Agent (10X)	1 µL	—
Deionized Water	to 10 µL	to 10 µL

2. Heat the sample for denaturing electrophoresis (reduced or non-reduced) at 85°C for 2 minutes for optimal results.

Prepare samples for non-denaturing electrophoresis

General guidelines

Non-denaturing (native) electrophoresis can be performed using NuPAGE™ Tris-Acetate, Novex™ Tris-Glycine, and Novex™ Tris-Glycine Plus Midi Gels.

- Do not add reducing agent to samples.
- **Do not heat samples for non-denaturing (native) electrophoresis.**

Materials needed

You will need the following items. See page 30 for ordering information.

- Protein sample and molecular weight marker
- Deionized water
- Novex™ Tris-Glycine Native Sample Buffer (2X)

Prepare samples

1. Use the volumes in the following table to prepare samples in a total volume of 10 µL. To prepare samples in other volumes, scale the volume of reagents accordingly. See page 6 for the recommended protein load.

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

Guidelines for running buffers

General guidelines

Non-denaturing (native) electrophoresis can be performed using NuPAGE™ Tris-Acetate, Novex™ Tris-Glycine, and Novex™ Tris-Glycine Plus Midi Gels.

- Do not use the NuPAGE™ Antioxidant with Novex™ Tris-Glycine or Tris-Glycine Plus Midi Gels.
- **Do not heat samples for non-denaturing (native) electrophoresis.**

Running buffers

Five types of running buffers are used for gel electrophoresis of Novex™ Midi Gels. See page 30 for ordering information.

Running buffer	Novex™ Midi Gels			
	NuPAGE™ Bis-Tris	NuPAGE™ Tris-Acetate	Tris-Glycine	Tris-Glycine Plus
Denaturing electrophoresis				
NuPAGE™ MES SDS Running Buffer (20X)	✓	–	–	–
NuPAGE™ MOPS SDS Running Buffer (20X)	✓	–	–	–
NuPAGE™ Tris-Acetate SDS Running Buffer (20X)	–	✓	–	–
Novex™ Tris-Glycine SDS Running Buffer (10X)	–	–	✓	✓
Non-denaturing electrophoresis				
Novex™ Tris-Glycine Native Running Buffer (10X)	–	✓	✓	✓

Amount of buffer required for the XCell4 SureLock™ Midi-Cell

The amount of 1X Running Buffer required will depend on the number of gels used in the apparatus as indicated below:

XCell4 *SureLock™* Midi-Cell	
Number of Gels	*Amount of Buffer*
4	1400 mL
3	1250 mL
2	950 mL
1	750 mL

Important

In the NuPAGE™ gels, 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.

Prepare running buffer for denaturing electrophoresis

Materials needed

- Deionized water
- NuPAGE™ Antioxidant for reduced samples with NuPAGE™ Midi Gels
- Appropriate SDS Running Buffer
  - NuPAGE™ MOPS or MES SDS Running Buffer (20X)
  - NuPAGE™ Tris-Acetate SDS Running Buffer (20X)
  - Novex™ Tris-Glycine SDS Running Buffer (10X)
- NuPAGE™ Antioxidant for reduced samples with NuPAGE™ Midi Gels

Prepare 1X NuPAGE™ Running Buffer

Use the volumes in the following table to prepare 1000 mL of **1X NuPAGE™ SDS Running Buffer**. Scale-up the volumes accordingly if more buffer is required.

Reducing Conditions

1. Prepare 1000 mL 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.
3. Immediately, prior to electrophoresis, add NuPAGE™ Antioxidant to 1X NuPAGE™ SDS Running Buffer from Step 1 for use in the Upper (Inner) Buffer Chamber. Mix thoroughly.
   - For the XCell4 *SureLock*™ Midi-Cell, add 435 µL NuPAGE™ Antioxidant to 175 mL 1X NuPAGE™ SDS Running Buffer.
   - For the Criterion™ Cell, add 150 µL NuPAGE™ Antioxidant to 60 mL 1X NuPAGE™ SDS Running Buffer

Non-Reducing Conditions

1. Prepare 1000 mL 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 use this buffer in the Lower (Outer) and Upper (Inner) Buffer Chambers.

Prepare 1X Tris-Glycine SDS Running Buffer

Use the volumes in the following table to prepare 1000 mL of **1X Tris-Glycine SDS Running Buffer**. Scale-up the volume of reagents accordingly if more buffer is required.

1. Prepare 1000 mL 1X Tris-Glycine SDS Running Buffer using Novex™ Tris-Glycine SDS Running Buffer (10X) as follows:

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

2. Mix thoroughly and use this buffer in the Lower (Outer) and Upper (Inner) Buffer Chambers.

Prepare running buffer for non-denaturing electrophoresis

Materials needed

You will need the following items. See page 30 for ordering information.

- Deionized water
- Novex™ Tris-Glycine Native Running Buffer (10X)

Prepare 1X Non-denaturing running nuffer

Instructions to prepare 1000 mL **1X Tris-Glycine Native Running Buffer** are described below. Scale-up the volume of reagents accordingly if more buffer is needed.

1. Prepare 1000 mL 1X Tris-Glycine Native Running Buffer using Novex™ Tris-Glycine Native Running Buffer (10X) as follows:

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

2. Mix thoroughly and use this buffer in the Lower (Outer) and Upper (Inner) Buffer Chambers.

Perform electrophoresis

Introduction

Instructions are provided below for electrophoresis of the Novex™ Midi Gels using the XCell4 *SureLock*™ Midi-Cell from Thermo Fisher Scientific (page 30).

For electrophoresis of the Novex™ Midi Gels using the Criterion™ Cell from Bio-Rad, see page 26.

Gels are individually packaged in clear pouches with Packaging Buffer. The Packaging Buffer contains low levels of residual acrylamide monomer and 0.02% sodium azide. Wear gloves 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 SDS, see page 32.

Materials needed

You will need the following items:

- Appropriate Novex™ Midi Gels (page 6)
- Protein sample (see page 12 for denaturing electrophoresis, or page 13 for non-denaturing electrophoresis)
- 1X Running Buffer (page 14)
- Gel loading tips
- XCell4 *SureLock*™ Midi-Cell (page 30)
- NuPAGE™ Antioxidant for reduced samples for use with NuPAGE™ gels
- Power Supply (e.g., PowerEase™ 300W Power Supply)

Note

Brief instructions for performing electrophoresis with 4 gels using the XCell4 *SureLock*™ Midi-Cell are described on page 17.

For detailed instructions for performing electrophoresis with less than 4 gels, see the XCell4 *SureLock*™ Midi Cell User Guide supplied with the Midi-Cell or access the manual online at **thermofisher.com**

Electrophoresis using the XCell4 *SureLock*™ Midi-Cell

Instructions for performing electrophoresis with **4 midi gels** using the XCell4 *SureLock*™ Midi-Cell are described below.

1. Remove the gel cassette from the pouch and rinse with deionized water.
2. Peel off the tape covering the slot on the back of the gel cassette and gently pull the comb out of the cassette. Rinse the wells with 1X Running Buffer and fill the sample wells with running buffer.
3. Insert the XCell4 *SureLock* ™ Assembly in its unlocked position into the center of the Midi-Cell base.
4. Place one gel cassette on each side of the Buffer Core for each of the cores.
5. While holding the assembly together with your hands, insert the Buffer Cores with gel cassettes into the Lower Buffer Chamber such that the negative electrode fits into the opening in the gold plate on the Lower Buffer Chamber. Always hold the assembly by its edges.
6. Lock the XCell4 *SureLock* ™ Assembly by moving the tension lever to the locked position (indicated on the XCell4 *SureLock* ™ Assembly). This will squeeze the gels and Buffer Cores together, creating leak free seals.
7. Fill each of the Upper Buffer Chambers with 175 mL of the appropriate 1X Running Buffer. For reducing conditions with NuPAGE™ Midi Gels, use 1X Running Buffer with 435 µL NuPAGE™ Antioxidant in each of the Upper Buffer Chambers. Ensure that the Upper Buffer Chambers are not leaking.
8. Load an appropriate volume of the protein 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 11 for recommended markers).
10. Add 700 mL 1X Running Buffer (for 4 gels) to the Lower Buffer Chamber (anode) by pouring into the center of the Midi-Cell (over the XCell4 *SureLock*™ Assembly). Fill to the fill line marked on the Midi-Cell.
11. Place the lid on the assembled XCell4 *SureLock* ™ Midi-Cell. The lid will firmly seat if the (–) and (+) electrodes are properly aligned.

Run conditions

Perform electrophoresis as described in the table below. Current readings are denoted per gel.

Note: Run times and currents are dependent on gel percentage and power supply.

Midi gel type and buffer system	Voltage	Expected current	Approximate run time
Denaturing electrophoresis			
Bis-Tris SDS-PAGE (MES Running Buffer)	200 V	Start: 160–200 mA End: 120–170 mA	40 minutes
Bis-Tris SDS-PAGE (MOPS SDS Running Buffer)	200 V	Start: 160–200 mA End: 120–170 mA	55 minutes
Tris-Acetate SDS-PAGE (Tris-Acetate SDS Running Buffer)	150 V	Start: 70–90 mA End: 50–60 mA	70 minutes
Tris-Glycine Plus (Tris-Glycine SDS Running Buffer)	200 V	Start: 70–75 mA End: 35–40 mA	55–65 minutes
Tris-Glycine (Tris-Glycine SDS Running Buffer)	125 V	Start: 40–50 mA End: 20–25 mA	105 minutes

Midi gel type and buffer system	Voltage	Expected current	Approximate run time
Non-denaturing electrophoresis			
Tris-Acetate (Tris-Glycine Native Running Buffer)	150 V	Start: 40–45 mA End: 15–20 mA	2–3 hours
Tris-Glycine Plus with Tris-Glycine Native Running Buffer	125 V	Start: 35–40 mA End: 15–20 mA	105–125 minutes
Tris-Glycine (Tris-Glycine Native Running Buffer)	125 V	Start: 35–40 mA End: 15–20 mA	115–125 minutes

Remove gel from cassette

1. After electrophoresis is complete, shut off the power, disconnect electrodes, and remove the lid.
2. Unlock the XCell4 *SureLock*™ Assembly by moving the tension lever to the unlocked position (indicated on the XCell4 *SureLock*™ Assembly).
3. Remove the Buffer Cores with the gel cassettes from the Lower Buffer Chamber while holding the cassettes against the cores. Remove the gel cassettes from the Buffer Cores and lay the gel cassettes on a flat surface, such as the bench top. The notched ("well") side of the cassette should face up.
4. Separate each of the three bonded sides of the cassette by inserting the Gel Knife into the gap between the cassette's two plates. 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.
5. Carefully remove and discard the top plate, allowing the gel to remain on the bottom (slotted) plate.

   Note: The Cassette Post (small plastic piece near the top of the cassette) may remain on either plate of the cassette after opening the two plates of the cassette. The Cassette Post is designed to maintain proper electrophoresis conditions that result in optimal separation and does not interfere with the sample loading or the electrophoresis run.
6. If blotting, remove the gel foot and well sections of the gel. Proceed immediately to western transfer (see page 22).
7. If staining, remove the gel from the plate by one of the following methods:
   - Use the sharp edge of the gel knife to remove the bottom foot 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 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 foot off of the gel after fixing or staining, but before gel drying.
8. Fix and stain the gel as described on page 19.

Visualizing bands in Novex™ Midi Gels

Compatible stains

Novex™ Midi Gels are compatible with most staining protocols including silver, Coomassie, and fluorescent stains. See page 30 for ordering details.

Silver staining

The SilverQuest™ Silver Staining Kit or the SilverXpress™ Silver Staining Kit are recommended for silver staining of Novex™ Midi Gels.

Coomassie staining

Novex™ Midi Gels are compatible with any of the standard Coomassie staining procedures. Protocols that are accelerated by heat are preferable as the heat serves to fix proteins, especially smaller peptides.

SimplyBlue™ SafeStain (see page 20 for a brief protocol) and Novex™ Colloidal Coomassie Blue Staining Kit are recommended for staining Novex™ Midi Gels.

Fluorescent staining

Novex™ Midi Gels are compatible with fluorescent stains such as the SYPRO™ Ruby Protein Gel Stain (see page 21 for a brief protocol).

General staining guidelines

You may use any staining protocol of choice. Follow the general guidelines listed below to obtain the best results:

- For converting a mini-gel staining protocol to stain the Novex™ Midi Gel, use ~1.5 times the volume of reagents recommended for a mini-gel.
- The volume of fixing, staining, and destaining solutions will depend on the volume of your staining container. To obtain good results, the solution volume must be sufficient to cover the gel completely and to allow the gel to move freely during all of the steps.
- When using a microwave oven for staining, be sure the gel is completely covered in the solution and use a microwaveable staining container. Use caution while using staining reagents in a microwave oven. Do not overheat the staining solutions.

Preservation of midi gels

The stained Midi Gels can be dried for storage or analysis by vacuum-drying or air-drying. We recommend using the Large Gel Drying Kit (page 30) to air-dry the gel.

SimplyBlue™ SafeStain protocol

Materials needed

- Incubation Trays (page 30) or appropriate staining containers
- Shaker
- Deionized water
- SimplyBlue™ SafeStain
- Microwave oven
- 20% NaCl (w/v) in deionized water

SimplyBlue™ SafeStain microwave protocol

SimplyBlue™ SafeStain (page 30) is a ready-to-use, proprietary Coomassie G-250 stain that is specially formulated for fast, sensitive detection and safe, non-hazardous disposal.

Instructions for staining gels with SimplyBlue™ SafeStain using a microwave oven are included in this section. For more details, refer to the SimplyBlue™ SafeStain manual available at **thermofisher.com** or contact Technical Support (page 32).

1. After electrophoresis, place the gel in 150 mL ultrapure water in a loosely covered microwaveable container and microwave on High (950–1100 watts) for 1 minute until the solution almost boils.

   Note: Do not use the Incubation Tray available from Thermo Fisher Scientific as the Incubation Tray is **not** heat-resistant and cannot be heated in a microwave oven or autoclaved.
2. Shake the gel on an orbital shaker for 1 minute. Discard the water.
3. Repeat Steps 1–2 two more times.
4. Add 40 mL SimplyBlue™ SafeStain and microwave on High for 45 seconds to 1 minute until the solution almost boils.
5. Shake the gel on an orbital shaker for 5 minutes. Discard the stain.
6. Wash the gel in 150 mL ultrapure water for 10 minutes on a shaker.
7. Add 30 mL 20% NaCl to the water in Step 6 and incubate for at least 5 minutes. The gel can be stored for several weeks in the salt solution.
8. *Optional:* Repeat Step 6 for 1 hour for a clear background.

SYPRO™ Ruby Protein Gel Stain protocol

Materials needed

- Incubation Trays (page 30) or appropriate staining containers
- Shaker
- Deionized water
- SYPRO™ Ruby Protein
- Fixing solution (20% acetic acid)
- Destaining solution (10% methanol, 7% acetic acid)
- UV transilluminator equipped with a standard camera or an appropriate laser scanner (page 21)

SYPRO™ Ruby staining protocol

SYPRO™ Ruby Protein Gel Stain (page 30) is a ready-to-use, highly sensitive fluorescent stain for protein staining.

Instructions for staining gels with SYPRO™ Ruby Protein Gel Stain are included in this section. For more details, refer to the SYPRO™ Ruby Protein Gel Stain manual available at **thermofisher.com** or contact Technical Support (page 32).

1. After electrophoresis, remove the gel from the cassette (page 18) and place the gel in a clean Incubation Tray.
2. Fix the gel in Staining Solution (20% acetic acid) for 30 minutes on an orbital shaker.
3. Stain the gel in undiluted SYPRO™ Ruby Protein Gel Stain for 1.5 hours on an orbital shaker.
4. Transfer the gel to a clean Incubation Tray and destain in Destaining Solution (10% methanol, 7% acetic acid) for ~2 hours. If complete removal of background is desired, perform the destaining step overnight.
5. Place the gel on a UV transilluminator equipped with a standard camera and select the ethidium bromide filter on the camera.

   You can also use a laser-based scanner with a laser line that falls within the excitation maxima of the stain (610 nm).
6. Image the gel with a suitable camera with the appropriate filters using a 1-4 second exposure. You may need to adjust the brightness and contrast to reduce any faint non-specific bands.

You should see fluorescent protein bands and the gel should have minimal background.

Western transfer

Compatible transfer systems

Novex™ Midi Gels can be blotted using any semi-dry or semi-wet transfer apparatus that can accommodate a a Novex™ Midi Gel (13 cm × 8.3 cm). A semi-dry blotting procedure for blotting Novex™ Midi Gels is described in the following section.

The gels are also compatible with the iBlot™ 2 Gel Transfer Device using a regular sized transfer stack (see page 24 for a brief protocol).

General guidelines

- Wear gloves at all times during the entire blotting procedure to prevent contamination of gels and membranes, and to avoid exposing your skin to irritants commonly used in electrophoresis and blotting procedures.
- Do not touch the membrane or gel with bare hands. This may contaminate the gel or membrane and interfere with further analysis.

Semi-dry blotting protocol

Materials needed

For ordering information, see page 30.

- Semi-dry transfer apparatus
- Methanol
- NuPAGE™ Transfer Buffer (20X) or Tris-Glycine Transfer Buffer (25X)
- NuPAGE™ Antioxidant (for use with NuPAGE™ gels)
- Blotting membranes: Invitrolon™/Filter Paper Sandwich or Nitrocellulose/Filter Paper Sandwiches
- 4 pieces of 2.5 mm thick Blotting Filter Paper per gel
- Blotting Roller
- Incubation Tray

Types of transfer buffer

Use appropriate transfer buffer based on the type of midi gel you are using.

	NuPAGE™ Bis-Tris	NuPAGE™ Tris-Acetate	Tris-Glycine [1]	Tris-Glycine Plus [1]
NuPAGE™ Transfer Buffer	✓	✓	–	–
Tris-Glycine Transfer Buffer	–	–	✓	✓

[1] Because Edman protein sequencing is inhibited by Tris, use the NuPAGE™ Transfer Buffer instead of Tris-Glycine Transfer Buffer if you plan to perform protein sequencing from a membrane.

Prepare 2X NuPAGE™ Transfer Buffer

Prepare 500 mL of **2X** NuPAGE™ Transfer Buffer with 10% methanol using the NuPAGE™ Transfer Buffer (20X) as follows:

NuPAGE™ Transfer Buffer (20X)	50 mL
NuPAGE™ Antioxidant (for reduced samples only)	0.5 mL
Methanol	50 mL
Deionized Water	to 500 mL

Prepare 2X Tris-Glycine Transfer Buffer

Prepare 500 mL of **2X** Tris-Glycine Transfer Buffer with 10% methanol using the Tris-Glycine Transfer Buffer (25X) as follows:

Tris-Glycine Transfer Buffer (25X)	40 mL
Methanol	50 mL
Deionized Water	to 500 mL

Equilibrate the gel

Equilibration of the gel in transfer buffer results in the removal of salts that may increase conductivity and heat during transfer. Perform equilibration for the recommended time, as longer equilibration can result in protein diffusion.

1. After electrophoresis, remove the gel from the cassette as described on page 18.
2. Equilibrate the Midi Gel in 100 mL of the appropriate 2X Transfer Buffer (see above for recipes) for 10 minutes on an orbital shaker.

Prepare blotting membrane

Nitrocellulose

1. Use pre-cut Nitrocellulose/Filter Paper Sandwich or cut nitrocellulose membrane to the appropriate size (13 cm × 8.3 cm).
2. Soak the membrane in a 2X Transfer Buffer (see above for recipe) for several minutes in the Incubation Tray.

PVDF

1. Use pre-cut Invitrolon™/Filter Paper Sandwich or cut PVDF membrane to the appropriate size (13 cm × 8.3 cm).
2. Pre-wet the membrane for 30 seconds in methanol, ethanol, or isopropanol. Briefly rinse the membrane in deionized water.
3. Soak the membrane in a 2X Transfer Buffer (see above for recipe) for several minutes in the Incubation Tray.

Transfer protocol

Instructions are provided below for blotting Tris-Glycine and Tris-Glycine Plus Midi Gels using a semi-dry blotting apparatus with a lower anode plate.

1. In a clean container or Incubation Tray, briefly soak 2 pieces of 2.5 mm thick Blotting Filter Paper (8.6 cm × 13.5 cm) in the appropriate 2X Transfer Buffer (see page 22). Several pieces of thinner blotting paper can be used to produce a stack of equivalent thickness.
2. Remove any air bubbles trapped between filter paper sheets using the Blotting Roller while the paper is still submerged in buffer.
3. Place the 2 pieces of pre-soaked 2.5 mm thick Blotting Filter Paper from Step 1 (or equivalent thickness of thinner filter paper) on the anode plate of a semi-dry blotting apparatus. Remove any air bubbles between the paper and plate with the Blotting Roller.
4. Place the pre-soaked blotting membrane on top of the filter paper stack and remove any air bubbles with the Blotting Roller.
5. Place the gel on top of the blotting membrane and remove any air bubbles with the Blotting Roller or a wet gloved finger.
6. Briefly soak 2 additional pieces of 2.5 mm thick Blotting Filter Paper in the appropriate 2X Transfer Buffer as was done in Step 1, and then gently place them on top of the gel.
7. Ensure that the filter paper sheets are aligned properly and flush with the gel/membrane sandwich. Remove any air bubbles with the Blotting Roller
8. Place the cathode plate on the stack without disturbing the blot sandwich. Follow the manufacturer's instructions to further assemble the semi-dry blotting apparatus.
9. Transfer at 20 V for 1 hour (~33 V/cm). You may need to optimize the transfer conditions for your specific proteins or semi-dry transfer apparatus.

iBlot™ 2 Gel Transfer Device protocol

Introduction

Brief instructions are provided below for blotting Novex™ Midi Gels using the iBlot™ 2 blotting apparatus. For detailed instructions for performing blotting, see the iBlot™ 2 Dry Blotting System User Guide or access the manual online at **thermofisher.com**

Equilibrate the gel

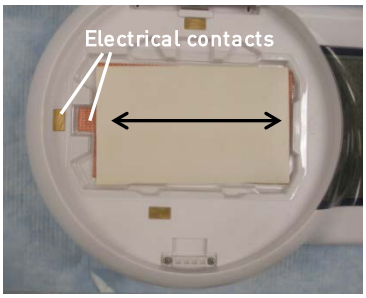
Equilibrating the gel in 100 mL of deionized water or transfer buffer for 5 minutes prior to transfer may improve transfer of mid to small molecular weight proteins (See step 7 in the following protocol).

This equilibration step can be omitted for optimal transfer of higher molecular weight proteins (>150kDa).

Transfer protocol

1. Select Method P0 on the iBlot™ 2 Gel Transfer Device.
2. Unseal an iBlot™ 2 Regular Size Transfer Stack.
3. Separate the Top Stack and set it to one side of the bench with the transfer gel layer facing up. **Keep the Bottom Stack in the transparent plastic tray.**
4. Place the Bottom Stack **with the plastic tray** directly on the blotting surface and align the electrical contacts on the tray with the corresponding electrical contacts on the blotting surface of the iBlot™ 2 Gel Transfer Device.

Orientation for iBlot® 2 **Regular** SizeTransfer Stack



5. Remove any air bubbles trapped between the membrane and transfer stack with the Blotting Roller.
6. Remove the gel from the cassette as described on page 18, and cut off the foot of the gel.
7. Immerse the gel in 100 mL of deionized water briefly (1–10 seconds) to facilitate easy positioning of the gel on top of the transfer membrane.

   Note: For Tris-Glycine Plus gels, rinse in 100 mL of deionized water for 5 minutes to improve transfer of mid to low molecular weight proteins (< 150 kDa).
8. Remove any air bubbles between the gel and membrane with the Blotting Roller.
9. Place a wet iBlot™ Filter Paper on top of the gel and remove any air bubbles with the Blotting Roller.
10. Place the Top Stack and palce it on top of the filter paper with the copper electrode facing up. Remove any air bubbles with the Blotting Roller.
11. Place the iBlot™ 2 Absorbent Pad on top of the transfer stack. Ensure the electrical contacts are aligned with the corresponding contacts on the blotting surface of the iBlot™ 2 Gel Transfer Device.
12. Flatten any protrusions on the surface of the stack with the Blotting Roller.
13. Close the lid of the iBlot™ 2 Gel Transfer Device gently.
14. Transfer using Method P0 on the iBlot™ 2 Gel Transfer Device.

Note: Blotting parameters (volts or time) may need optimization based on your initial results.

Appendix A

Troubleshooting

Introduction Review the information below to troubleshoot your experiments with Novex™ Midi Gels.

Observation	Cause	Solution
Run taking longer time	Running buffer too dilute	• Ensure the running buffer corresponds to the type of gel being used (page 14). • Make fresh running buffer as described on page 15 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 gel 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. • Ensure the lid is properly positioned and seated correctly.
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.
Poor resolution, bands are not very sharp (fuzzy, smeary, streaking)	Incorrect sample or running buffer used	Use the recommended sample buffer and 1X Running Buffer based on the gel type. Do not use the NuPAGE™ Bis-Tris Midi Gels with NuPAGE™ MOPS or MES Running Buffer without SDS for native gel electrophoresis. **Do not** use NuPAGE™ SDS Running Buffer for electrophoresis of Tris-Glycine or Tris-Glycine Plus Midi Gels and **do not** use Tris-Glycine SDS Running Buffer for electrophoresis of NuPAGE™ Midi Gels.
High background staining in upper portion of gel stained with Simply Blue SafeStain™	Higher staining of low % acrylamide portion of the gel	Add 5 mL 20% NaCl (w/v in deionized water) to the gel rinse after the initial water destain (1 hour at room temperature if not using the microwave protocol). Continue to destain for at least 5 minutes, or until the desired low background is produced.

Appendix B

Using Novex™ Midi Gels with the Criterion™ Cell

Introduction

To efficiently use the Novex™ Midi Gels with the Criterion™ Cell from Bio-Rad, you will need to use the Midi Gel Adapter with the Novex™ Midi Gel. The Midi Gel Adapter is supplied with the Novex™ Midi Gels with Adapters as well as available separately.

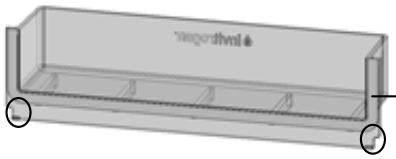
Brief instructions for using the Novex™ Midi Cassette with a Midi Gel Adapter for use with the Criterion™ Cell are described in this section. For details on using the Criterion™ Cell, refer to the manual supplied with the apparatus.

To use a Novex™ Midi Gel with the XCell4 *SureLock*™ Midi-cell, see page 17.

Note: During electrophoresis, it is normal for electrical current values to be slightly higher and run times to be slightly shorter for a given run voltage than when the XCell4 Surelock Midi-Cell is used.

Midi Gel Adapter

Each Midi Gel Adapter is designed with two alignment tabs (indicated with circles in the figure below) that fit into the slots on the Novex™ Midi Cassette and facilitate the attachment of the adapter onto the Novex™ Midi Cassette. The Midi Gel Adapter contains an adhesive on the inner side. After removing the adhesive liner from the adapter and placing the adapter on a **dry** surface of the Novex™ Midi Cassette, the adhesive creates a tight seal between the adapter and cassette and holds each adapter on to the cassette.



The Novex™ Midi Cassette / Adapter assembly makes the Novex™ Midi Gel compatible for use with the Criterion™ Cell and creates an upper buffer chamber that can hold ~75 mL of running buffer for electrophoresis.

Note

- The Midi Gel Adapter is designed for use with the Novex™ Midi Gel in the Criterion™ Cell (Bio-Rad) **only**. Do not use the Midi Gel Adapter with any other electrophoresis apparatus.
- **Do not** re-use the Midi Gel Adapter. Discard the adapter after use.

Materials needed

You will need the following items. For ordering information, see page 30.

- Novex™ Midi Gels with Adapters
- Protein sample prepared in the appropriate sample buffer (see page 12 for denaturing electrophoresis, or page 13 for non-denaturing electrophoresis)
- Appropriate 1X Running Buffer (see page 14)
- Gel loading tips
- Criterion™ Cell (available from Bio-Rad)
- NuPAGE™ Antioxidant for reduced samples for use with NuPAGE™ gels
- Power Supply (e.g., PowerEase™ 300W Power Supply)

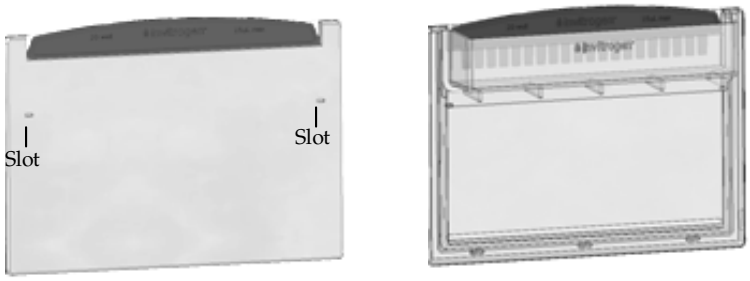
Important

- To obtain a tight seal, be sure to insert the alignment tabs of the adapter into the two slots of the cassette and press the adapter firmly on the cassette.
- Leaks are generally caused:
  - When the adapter is not firmly pressed onto the cassette or the surface of the cassette was wet when the adapter was applied. **To obtain a tight seal, press the adapter firmly on the cassette.**
  - By adding excess buffer. The Midi Gel Adapter is designed to hold ~75 mL buffer.
- When leaks occur, it is best to remove the adapter and discard it. Remove any remaining adhesive on the cassette, dry the cassette with a paper towel and then place a fresh new adapter on the **dried** surface of the cassette as described in the following section.

Attach the midi gel adapter

1. Remove one Midi Gel Adapter and one Novex™ Midi Gel from their individual packages.
2. **After ensuring that your hands are dry, blot any excess liquid from the cassette using a paper towel.**
3. Locate the slots on the Novex™ Midi Cassette as shown in figure A below. Avoid introducing any liquid onto the cassette surface.
4. Peel off the Adhesive Liner from the Midi Gel Adapter with dry hands.
5. Hold the Midi Gel Adapter such that the Invitrogen logo is facing towards you (adhesive side towards the cassette) and align the alignment tabs of the adapter with the two slots on the cassette. **Place the adapter on the cassette and apply firm pressure to the adapter on the adhesive area to ensure a tight seal between the adapter and cassette (figure B).**

Figure A (Cassette only) **Figure B (Cassette with Adapter)**



The attachment of the adapter on the gel cassette generates an **upper buffer chamber** that can hold ~75 mL running buffer and is required for use with the Criterion™ Cell.

6. Remove the comb from the cassette and rinse the wells with 1X Running Buffer.
7. Remove the tape from the bottom of the cassette.
8. Use the Novex™ Midi Cassette/Adapter assembly immediately for electrophoresis as described on page 28. We recommend using the cassette/adapter assembly within 1 hour of assembly to obtain the best results and prevent any leaks.

Amount of buffer

The amount of 1X Running Buffer required will depend on the number of gels used in the apparatus as indicated below:

For Criterion™ Cell (Bio-Rad)	
Number of gels	*Amount of buffer*
2	1000 mL
1	500 mL

Perform electrophoresis

1. Insert the Novex™ Midi Cassette / Adapter assembly into one of the slots in the Criterion™ Cell tank such that the adapter is facing the center of the cell.
2. Add 60 mL of the appropriate 1X Running Buffer into the upper buffer chamber. For reduced samples being run on NuPAGE™ gels, use 60 mL 1X Running Buffer containing 150 µL NuPAGE™ Antioxidant. If you notice any leaks, see page 26.
3. Load the appropriate volume of samples and protein molecular weight markers in the wells.
4. Load each half of the lower buffer chamber with 400 mL 1X Running Buffer (for electrophoresis of 2 gels).
5. Place the lid on the Criterion™ Cell.
6. With the power off, connect the electrode cords to the power supply. Turn on the power supply and perform electrophoresis using the following settings:

Midi gel type and buffer system	Voltage	Expected current	Approximate run time
NuPAGE™ Novex™ Bis-Tris with MES SDS Running Buffer (denaturing, reducing)	200 V	Start: 250–270 mA End: 150–170 mA	35 minutes
NuPAGE™ Novex™ Bis-Tris with MOPS SDS Running Buffer (denaturing, reducing)	200 V	Start: 250–270 mA End: 150–170 mA	40 minutes
NuPAGE™ Novex™ Tris-Acetate with Tris-Acetate SDS Running Buffer (denaturing, reducing)	150 V	Start: 80–100 mA End: 50–60 mA	60 minutes
NuPAGE™ Novex™ Tris-Acetate with Tris-Glycine Native Running Buffer (non-denaturing)	150 V	Start: 50–60 mA End: 15–20 mA	1.5–2 hours
Tris-Glycine with Tris-Glycine SDS Running Buffer	125 V	Start: 55–70 mA End: 20–30 mA	90 minutes (depending on gel type)
Tris-Glycine with Tris-Glycine Native Running Buffer (non-denaturing)	125 V	Start: 45–55 mA End: 15–20 mA	100 minutes (depending on gel type)
Tris-Glycine Plus with Tris-Glycine SDS Running Buffer	200 V	Start: 95–105 mA End: 35–50 mA	45–55 minutes
Tris-Glycine Plus with Tris-Glycine Native Running Buffer (native electrophoresis)	125 V	Start: 50–60 mA End: 15–20 mA	90–105 minutes

Note: Run times and currents are dependent on gel percentage and power supply

7. Disassemble the Criterion™ Cell as described in the manual supplied with the apparatus.

Appendix C

Novex™ Midi Gel specifications

Specifications

Gel Matrix:	Acrylamide/Bisacrylamide
Gel Size:	13 cm × 8.3 cm
Gel Thickness:	1.0 mm
Cassette Size:	15 cm × 10.3 cm
Cassette Material:	Styrene Copolymer
Gel Types:	NuPAGE™ Bis-Tris, NuPAGE™ Tris-Acetate, Novex™ Tris-Glycine, Novex™ Tris-Glycine Plus
Sample Well Configuration:	12+2, 20, and 26 well

Appendix D

Accessory products

Additional products

Ordering information for electrophoresis products available separately from Thermo Fisher Scientific is provided below. For detailed information, visit our website at **thermofisher.com** or call Technical Support (page 32).

Product	Quantity	Catalog No.
XCell4 *SureLock*™ Midi-Cell	1 unit	WR0100
NuPAGE™ Tris-Acetate SDS Buffer Kit	1 kit	LA0050
NuPAGE™ Tris-Acetate SDS Running Buffer (20X)	500 mL	LA0041
NuPAGE™ Sample Reducing Agent (10X)	250 µL	NP0004
NuPAGE™ Antioxidant	15 mL	NP0005
NuPAGE™ LDS Sample Buffer (4X)	10 mL	NP0007
NuPAGE™ Transfer Buffer (20X)	1 L	NP0006-1
NuPAGE™ MOPS SDS Buffer Kit	1 kit	NP0050
NuPAGE™ MES SDS Buffer Kit	1 kit	NP0060
NuPAGE™ MOPS SDS Running Buffer (20X)	500 mL	NP0001
NuPAGE™ MES SDS Running Buffer (20X)	500 mL	NP0002
Novex™ Tris-Glycine SDS Sample Buffer (2X)	20 mL	LC2676
Novex™ Tris-Glycine SDS Running Buffer (10X)	500 mL	LC2675
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
Stains	**Quantity**	**Catalog No.**
SimplyBlue™ SafeStain	1 L	LC6060
SilverQuest™ Silver Staining Kit	1 kit	LC6070
SilverXpress™ Silver Staining Kit	1 kit	LC6100
SYPRO™ Ruby Protein Gel Stain	1 L	S-12000
Large Gel Drying Kit	1 kit	NI2207
Protein standards	**Quantity**	**Catalog No.**
PageRuler™ Plus Prestained Protein Ladder	2 × 250 µL	26619
PageRuler™ Prestained Protein Ladder	2 × 250 µL	26616
NativeMark™ Unstained Protein Ladder	5 × 50 µL	LC0725
HiMark™ Pre-Stained HMW Protein Standard	250 µL	LC5699
HiMark™ Unstained HMW Protein Standard	250 µL	LC5688
SeeBlue™ Plus2 Pre-Stained Standard	500 µL	LC5925
BenchMark™ Protein Ladder	2 × 250 µL	10747-012
MagicMark™ XP Western Protein Standard	250 µL	LC5602
Mark12™ Unstained Standard	1 mL	LC5677

Blotting Products	Quantity	Catalog no.
Nitrocellulose/Filter Paper Sandwich, 0.45 µm	16/pack	LC2006
Nitrocellulose/Filter Paper Sandwich, 0.2 µm	16/pack	LC2009
Invitrolon™/Filter Paper Sandwich, 0.45 µm	16/pack	LC2007
Blotting Filter Paper (2.5 mm thick)	50/pack	LC2008
Blotting Roller	1 each	LC2100
Incubation Tray	8/pack	LC2102
WesternBreeze™ Chromogenic Kit, Anti-Mouse	1 kit	WB7103
WesternBreeze™ Chromogenic Kit Anti-Rabbit	1 kit	WB7105
WesternBreeze™ Chemiluminescent Kit, Anti-Mouse	1 kit	WB7104
WesternBreeze™ Chemiluminescent Kit, Anti-Rabbit	1 kit	WB7106
Power Supply	**Quantity**	**Catalog no.**
PowerEase™ 300W Power Supply	1 unit	PS0300

Appendix E

Documentation and support

Obtaining support

For the latest services and support information for all locations, go to **thermofisher.com**

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support (**techsupport@lifetech.com)**
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches