NuPAGE™ Bis-Tris Midi Gels, WedgeWell™ Format

Cat Nos. WBT010XBOX, WBT412XBOX

QUICK REFERENCE Pub. No. MAN1000410 Rev. A

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Contents and storage

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Gel type	Amount	Storage
NuPAGE™ Bis-Tris Midi WedgeWell™ Gels	Box of 10 gels	Store at 4–25°C for up to 12 months. Do not freeze.

Product description

NuPAGE™ Bis-Tris Midi WedgeWell™ Gels are precast polyacrylamide gels designed for optimal separation and resolution of small- to medium-sized proteins (1.5–300 kDa) under denaturing conditions. NuPAGE™ Bis-Tris Midi Gels have a neutral pH environment that minimizes protein modifications. The gels feature wedge-shaped sample wells with a capacity of up to 100 µL of sample per well.

NuPAGE™ Bis-Tris Midi WedgeWell™ Gels are available with the following specifications:

- **Polyacrylamide percentage**: 10% and 4–12%
- **Well format**: 12+2, 20, and 26 wells
- **Thickness**: 1.0 mm

Required materials

- Protein sample and protein ladder
- NuPAGE™ MES or MOPS SDS Running Buffer (20X)
- NuPAGE™ LDS Sample Buffer (4X) (Cat. No. NP0007)
- NuPAGE™ Sample Reducing Agent (10X) (Cat. No. NP0009) for reduced samples
- NuPAGE™ Antioxidant (Cat. No. NP0005) for reduced samples
- SureLock™ Tandem Midi Gel Tank (Cat. No. STM1001) or XCell4 SureLock™ Midi-Cell Gel Running Tank (Cat. No. WR0100)
- Novex™ Power Supply Adapters (Cat. No. ZA10001) if not using a Thermo Fisher Scientific power supply

Online resources

- Visit thermofisher.com/proteingels for additional information and protocols.
- For support, visit thermofisher.com/support.

For Research Use Only. Not for use in diagnostic procedures.

Choosing a well format

Well type	Recommended loading volume	Maximum loading volume
12+2-well	90 +30 (small well) µL	100 + 35 (small well) µL
20-well	50 µL	60 µL
26-well	30 µL	40 µL

Choosing a protein ladder for your application

Type	Marker	Cat. No.
Pre-Stained	PageRuler™ Prestained Protein Ladder PageRuler™ Plus Prestained Protein Ladder	26616 26619
Unstained	PageRuler™ Unstained Protein Ladder PageRuler™ Unstained Broad Range Protein Ladder	26614 26630
Western blot	iBright™ Prestained Protein Ladder MagicMark™ XP Western Protein Standard	LC5615 LC5602

Go to thermofisher.com/proteinladders for more information on protein ladders.

Choosing buffers for your application

Buffer	Application	Cat. No.
NuPAGE™ MES SDS Running Buffer (20X)	Resolve small- to medium-size proteins	NP0002
NuPAGE™ MOPS SDS Running Buffer (20X)	Resolve medium- to large-size proteins	NP0001
NuPAGE™ Transfer Buffer (20X)	Wet transfer	NP0006

Life Technologies | 5781 Van Allen Way | Carlsbad, CA 92008

For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

The information in this guide is subject to change without notice.

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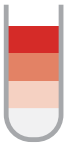
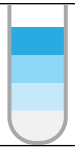
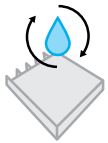
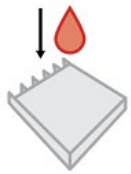
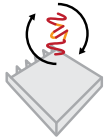
Perform denaturing protein gel electrophoresis using NuPAGE™ Bis-Tris Midi WedgeWell™ Gels

<table>
<tr><th colspan="2">Step</th><th>Action</th></tr>
<tr><td>1</td><td>  </td><td>Prepare samples</td><td>Prepare 1X Sample Buffer for dilutions of samples if needed. Volumes are provided for a 10-µL sample size. Scale volumes proportionally for larger sample sizes.
<table><tr><th>Components</th><th>Reduced sample</th><th>Non-reduced sample</th></tr><tr><td>Sample</td><td>x µL</td><td>x µL</td></tr><tr><td>NuPAGE™ LDS Sample Buffer (4X)</td><td>2.5 µL</td><td>2.5 µL</td></tr><tr><td>NuPAGE™ Sample Reducing Agent (10X)</td><td>1 µL</td><td>—</td></tr><tr><td>Deionized Water</td><td>to 6.5 µL</td><td>to 7.5 µL</td></tr><tr><td>Total Volume</td><td>10 µL</td><td>10 µL</td></tr></table>Heat samples at 70°C for 10 minutes.</td></tr>
<tr><td>2</td><td>  </td><td>Prepare buffers</td><td>Add 50 mL of 20X NuPAGE™ MES or MOPS Running Buffer to 950 mL of deionized water to prepare 1X SDS Running Buffer.
For reduced samples, add 1 mL of NuPAGE™ Antioxidant to 400 mL of 1X SDS Running Buffer.</td></tr>
<tr><td>3</td><td>  </td><td>Prepare gel</td><td>a. Remove the comb and rinse the gel wells three times using 1X Running Buffer.
b. Remove the white tape near the bottom of the gel cassettes.
c. Place the gels in the midi gel tank.</td></tr>
<tr><td>4</td><td>  </td><td>Load buffers</td><td>Fill the chambers with the appropriate 1X running buffer.
SureLock™ Tandem Midi Gel Tank: Add ~170 mL of buffer to cathode (inner) chamber. Fill the anode (outer) chamber to fill line (~350 mL).
XCell4 SureLock™ Midi-Cell: Add 175 mL of buffer to the cathode (inner) chambers. Fill the anode (outer) chambers to fill line.
For reduced samples, use running buffer with antioxidant in the cathode chambers.</td></tr>
<tr><td>5</td><td>  </td><td>Load samples and ladders</td><td>a. Load the appropriate volume of your samples in the appropriate wells.
b. Load your protein ladder in the appropriate well.</td></tr>
<tr><td>6</td><td>  </td><td>Run the gel</td><td>Run times vary depending on gel percentage, power supply, and electrophoresis device used.
If using MES running buffer, run for 25–35 minutes at 200 V constant.
If using MOPS running buffer, run for 50–60 minutes at 200 V constant.
Note: If you are not using a Thermo Fisher Scientific™ power supply, install Novex™ Power Supply Adapters</td></tr>
</table>

Buffer formulation

The following recipes are provided to allow preparation of buffers from scratch.

The pH listed for each buffer is for the 1X solution. **Do not use acid or base to adjust the pH**. Buffers are stable for 6 months when stored at 4°C.

Prepare 500 mL of 20X MES SDS Running Buffer

50 mM MES, 50 mM Tris Base, 0.1% SDS, 1 mM EDTA, pH 7.3

1. Dissolve the following reagents in 400 mL of ultrapure water.

Reagent	Amount
MES	97.6 g
Tris Base	60.6 g
SDS	10.0 g
EDTA	3.0 g

2. Mix well and adjust the volume to 500 mL with ultrapure water.
3. Before electrophoresis, dilute buffer to 1X with water.

Prepare 500 mL of 10X MOPS SDS Running Buffer

50 mL MOPS, 50 mM Tris Base, 0.1% SDS, 1 mM EDTA, pH 7.7

1. Dissolve the following reagents in 400 mL of ultrapure water.

Reagent	Amount
MOPS	104.6 g
Tris Base	60.6 g
SDS	10.0 g
EDTA	3.0 g

2. Mix well and adjust the volume to 1,000 mL with ultrapure water.
3. Before electrophoresis, dilute buffer to 1X with water.

Prepare 125 mL of 20X Bis-Tris Transfer Buffer

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

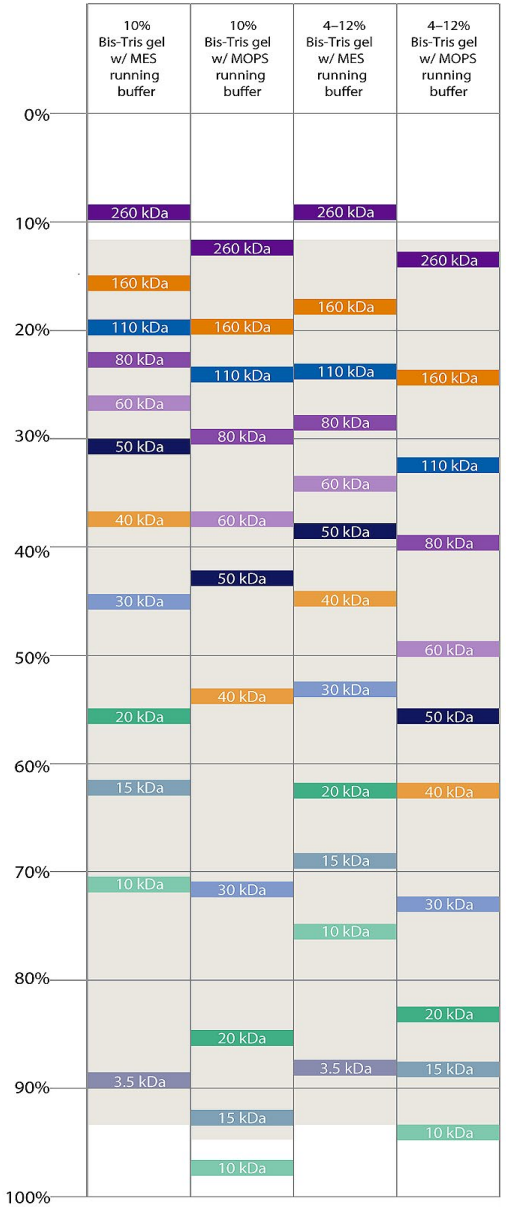
1. Dissolve the following reagents in 100 mL of ultrapure water.

Reagent	Amount
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. Before western transfer, dilute buffer to 1X with water.

Migration patterns of protein standards on NuPAGE™ Bis-Tris gels

Refer to the migration chart to find the gel best suited for your application. Your proteins of interest should migrate through ~70% of the length of the gel for the best resolution.



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