NuPAGE™ Bis-Tris Mini Gels

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QUICK REFERENCE Pub. No. MAN0007891 Rev. D

Contents and storage

Gel type	Amount	Storage
NuPAGE™ Bis-Tris Gels	Box of 2 or 10 gels	Store at 4–25°C for up to 1 year. Do not freeze.

Product description

NuPAGE™ Bis-Tris Gels are precast polyacrylamide gels designed for optimal separation and resolution of small- to medium-sized proteins (1.5–300 kDa) under denaturing gel electrophoresis conditions.

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

- **Polyacrylamide percentage**: 10%, 12%, and 4–12%
- **Well format**: 1, 9, 10, 12, 15, 17, IPG, and 2D wells
- **Thickness**: 1.0 mm and 1.5 mm

Required materials

- Protein sample and protein ladder
- NuPAGE™ **MES** or **MOPS** SDS Running Buffer (20X)
- NuPAGE™ LDS Sample Buffer (4X)
- NuPAGE™ Sample Reducing Agent (10X) (for reduced samples)
- NuPAGE™ Antioxidant (for reduced samples)
- Novex™ Power Supply Adapters (Cat. No. ZA10001) if not using a Thermo Fisher Scientific™ power supply
- Mini Gel Tank (Cat. No. A25977) or XCell SureLock™ Mini-Cell (Cat. No. EI0001)

Online resources

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

Choosing a well format

Thicker 1.5 mm gels with fewer wells are recommended for large samples (>30 µL). Thinner 1 mm gels are recommended for blotting because of better protein transfer.

Well type	Maximum loading volume [1]		Maximum protein load
	1 mm thickness	1.5 mm thickness	
1-well	700 µL	—	12 µg/band
IPG-well	7-cm IPG strip	—	—
2D-well	400 µL	600 µL	12 µg/band
9-well	28 µL	—	0.5 µg/band
10-well	25 µL	37 µL	0.5 µg/band
12-well	20 µL	—	0.5 µg/band
15-well	15 µL	25 µL	0.5 µg/band
17-well	15 µL	—	0.5 µg/band

[1] Not every format is available for every gel type.

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™ MOPS SDS Running Buffer	Resolve mid-size proteins	NP0001
NuPAGE™ MES SDS Running Buffer	Resolve small molecular weight proteins	NP0002
NuPAGE™ Transfer Buffer	Wet transfer	NP0006

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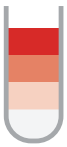
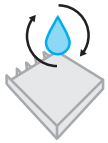
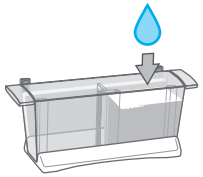
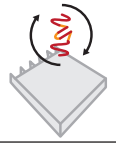
Perform denaturing protein gel electrophoresis using NuPAGE™ Bis-Tris Mini Gels

<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™ 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 [1]</td><td>10 µL [1]</td></tr></table>[1] See "Choosing a well format" for recommended loading volumes.
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 SDS 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 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 mini gel tank.</td></tr>
<tr><td>4</td><td>  </td><td>Load buffers</td><td>Fill the chambers with the appropriate 1X running buffer.
Mini Tank: Add 400 mL of buffer to each chamber.
XCell SureLock™ Mini-Cell: Add 600 mL of buffer to the lower chamber, and 200 mL to the upper chamber (for reduced samples, use running buffer with antioxidant in the upper chamber).</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>Optimal run times vary depending on gel percentage and power supply used for electrophoresis.
If using MES Running Buffer, run for 35 minutes at 200 V constant.
If using MOPS Running Buffer, run for 50 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 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 20X MOPS SDS Running Buffer

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

1. Dissolve the following reagents in 400 mL 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 500 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

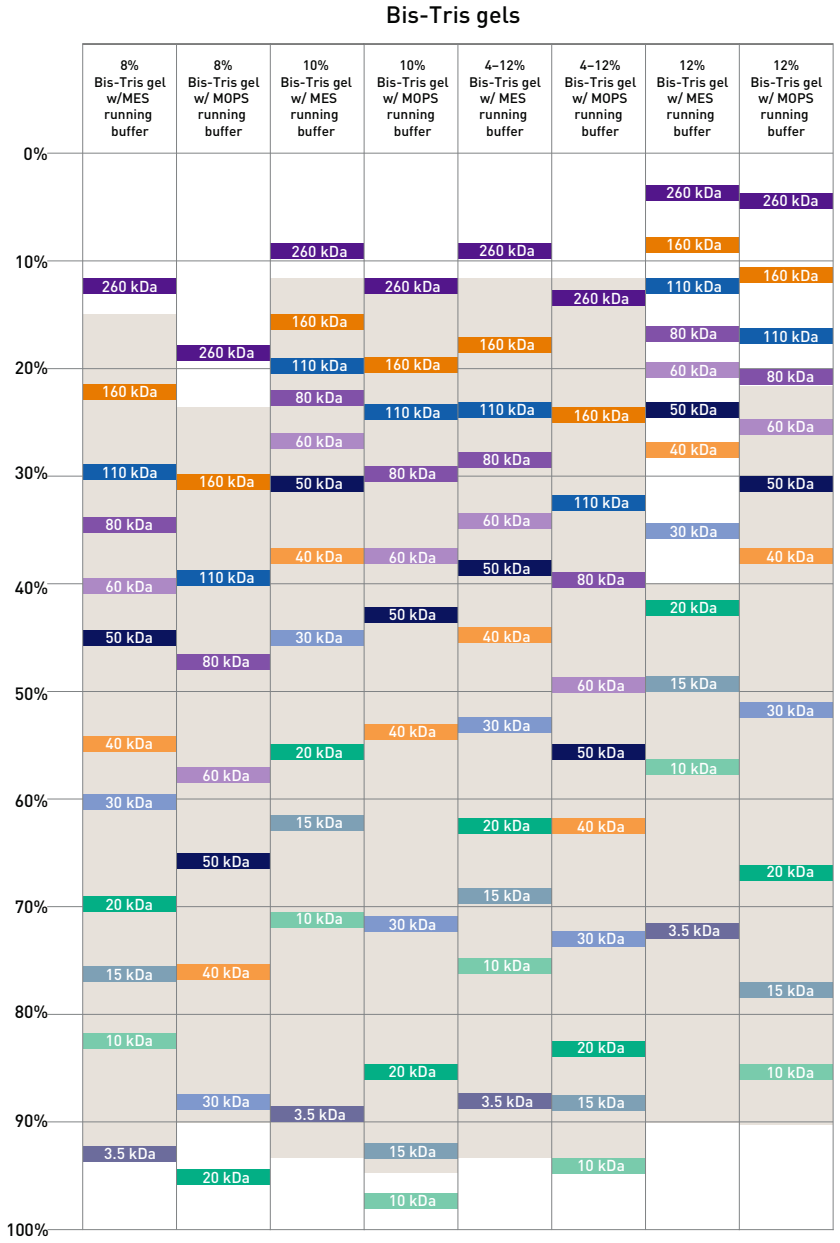
1. Dissolve the following reagents in 100 mL ultrapure water.

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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