NuPAGE™ Bis-Tris Mini Gels

Pub. No. MAN0007891 Rev. E

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

Product description

NuPAGE™ Bis-Tris Gels are ready-to-use polyacrylamide gels specifically engineered for the effective separation and resolution of small to medium-sized proteins (ranging from 1.5 to 300 kDa) in denaturing gel electrophoresis.

Specifications of NuPAGE™ Bis-Tris Gels include:

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

Contents and storage

Item	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.

Required materials

Unless otherwise indicated, all materials are available through **thermofisher.com**.

- Protein sample and protein ladder
- NuPAGE™ Antioxidant (For reduced samples) (Cat. No. NP0005)
- NuPAGE™ Sample Reducing Agent, 10X (For reduced samples) (Cat. No. NP0004)
- NuPAGE™ LDS Sample Buffer, 4X (Cat. No. NP0007)
- 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)

Note: Visit **thermofisher.com/proteingels** for additional information and protocols.

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]	
	1 mm thickness	1.5 mm thickness
1-well	700 µL	—
IPG-well	7-cm IPG strip	—
2D-well	400 µL	600 µL
9-well	28 µL	—
10-well	25 µL	37 µL
12-well	20 µL	—
15-well	15 µL	25 µL
17-well	15 µL	—

[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

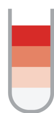
Note: Go to **http://thermofisher.com/proteinladders** for more information on protein ladders.

Choosing buffers for your application

Buffer	Application	Cat. No.
NuPAGE™ MOPS SDS Running Buffer (20X Liquid)	Resolve mid-size proteins	NP0001
NuPAGE™ MES SDS Running Buffer (20X Liquid)	Resolve small molecular weight proteins	NP0002
NuPAGE™ MOPS SDS Running Buffer (Powder Packet for 1 L)	Resolve mid-size proteins	NP000205
NuPAGE™ MES SDS Running Buffer (Powder Packet for 1 L)	Resolve small molecular weight proteins	NP000105

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

1 Prepare samples



Prepare 1X Sample Buffer for sample dilutions if necessary. Use the provided volumes for a 10 µL sample size and scale them proportionally for larger sample sizes.

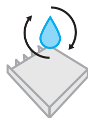
Components	Reduced sample	Non-reduced sample
Sample	x µL	x µL
NuPAGE™ LDS Sample Buffer (4X)	2.5 µL	2.5 µL
NuPAGE™ Reducing Agent (10X)	1 µL	–
Deionized Water	to 6.5 µL	to 7.5 µL
Total Volume	10 µL	10 µL

Note: Heat samples at 70°C for 10 minutes. Refer to "Choosing a well format" on page 1 for recommended loading volumes.

2 Prepare buffers

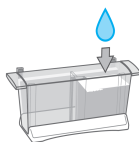
1. Add 50 mL of 20X NuPAGE™ MES or MOPS SDS Running Buffer to 950 mL of deionized water to prepare 1X SDS Running Buffer.
2. Alternatively, dissolve one packet of dry MES or MOPS SDS Running Buffer into 1000 mL of deionized water.
3. (Optional) For reduced samples, add 1 mL of NuPAGE™ Antioxidant to 400 mL 1X SDS Running Buffer.

3 Prepare gel



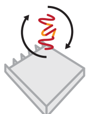
1. Remove the comb and rinse the gel wells three times using 1X Running Buffer.
2. Remove the white tape at the bottom of the gel cassettes.
3. Place the gels in the mini gel tank.

4 Load buffers



1. Fill the chambers with the appropriate 1X Running Buffer.
2. Mini Gel Tank: Add 400 mL of buffer to each chamber.
3. 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).

5 Load samples and ladders



1. Load the appropriate volume of your samples in the appropriate wells.
2. Load your protein ladder in the appropriate well.

6 Run the gel

1. Optimal run times vary depending on gel percentage and power supply used for performing electrophoresis.
2. Run for 24 minutes at a constant 200 V if using MES Running Buffer.
3. Run for 28 minutes at a constant 200 V if using MOPS Running Buffer.

   Note: If you are not using a Thermo Fisher Scientific power supply, install Novex™ Power Supply Adapters.

Buffer formulation

IMPORTANT! The below procedures are listed to enable the preparation of buffers from basic ingredients.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

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 (Final concentration: 50 mM MES, 50 mM Tris Base, 0.1% SDS, 1 mM EDTA, pH 7.3).

Prepare 500 mL of 20X MOPS SDS Running Buffer

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 (Final concentration: 50 mM MOPS, 50 mM Tris Base, 0.1% SDS, 1 mM EDTA, pH 7.3).

Prepare 125 mL of 20X Bis-Tris Transfer Buffer

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 (Final concentration: 25 mM Bicine, 25 mM Bis-Tris (free base), 1 mM EDTA, pH 7.2).

Prepare 1 L of 1X MOPS or MES Dry SDS Running Buffer

1. Dissolve one dry buffer pack in 1000 mL of ultrapure water.

   Note: Open carefully to avoid spillage.
2. Ensure all dry reagents have dissolved by mixing well.

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

Refer to the migration chart, to identify the most suitable gel for your application.

Limited product warranty

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

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