NuPAGE™ Bis-Tris Midi Gels, WedgeWell™ Format

Pub. No. MAN1000410 Rev. B

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. NuPAGE™ Bis-Tris Midi WedgeWell™ Gels have a neutral pH environment that minimizes protein modifications.

Specifications of NuPAGE™ Bis-Tris WedgeWell™ Midi Gels include:

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

Use the NuPAGE™ MES SDS Running Buffer for small proteins or NuPAGE™ MOPS SDS Running Buffer for medium-size proteins.

Contents and storage

Item	Amount	Storage
NuPAGE™ Bis-Tris Midi Gels, WedgeWell™ Format	Box of 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™ MES or MOPS SDS Running Buffer
- 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
- SureLock™ Tandem Mini Gel Tank (Cat. No. STM1001) or XCell4 SureLock™ Midi-Cell Gel Running Tank (Cat. No. WR0100)

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

Choosing a well format

Well type	Maximum loading volume
12+2-well	100 µL (sample) + 35 µL (marker)
20-well	60 µL
26-well	40 µL

Choosing a protein ladder for your application

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

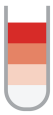
Note: Go to http://thermofisher.com/proteinladders for more information on protein ladders.

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™ Transfer Buffer	Wet transfer	NP0006
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 Midi 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.

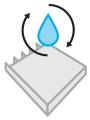
Components	Reduced sample	Non-reduced sample
Sample	x µL	x µL
NuPAGE™ LDS Sample Buffer (4X)	2.5 µL	2.5 µL
NuPAGE™ Reducing Agent (10X)	1 µL	–
Deionized Water	to 6.5 µL	to 7.5 µL
Total Volume	10 µL	10 µL

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

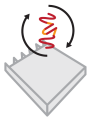


1. Remove the comb and rinse the gel wells three times using 1X Running Buffer.
2. Remove the white tape at the bottom of the gel cassettes.
3. Place the gels in the mini gel tank.

4 Load buffers

1. Fill the chambers with the appropriate 1X Running Buffer.
2. SureLock™ Tandem Mini Gel Tank: Add approximately 170 mL of buffer to cathode (inner) chamber. Fill the anode (outer) chamber to fill line (approximately 350 mL).
3. XCell 4 SureLock™ Mini-Cell: Fill each upper buffer chamber with 175 mL and lower buffer chamber to the fill line with the appropriate 1X Running Buffer.

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 25–35 minutes at a constant 200 V if using MES Running Buffer.
3. Run for 40–60 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 7.7).

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).

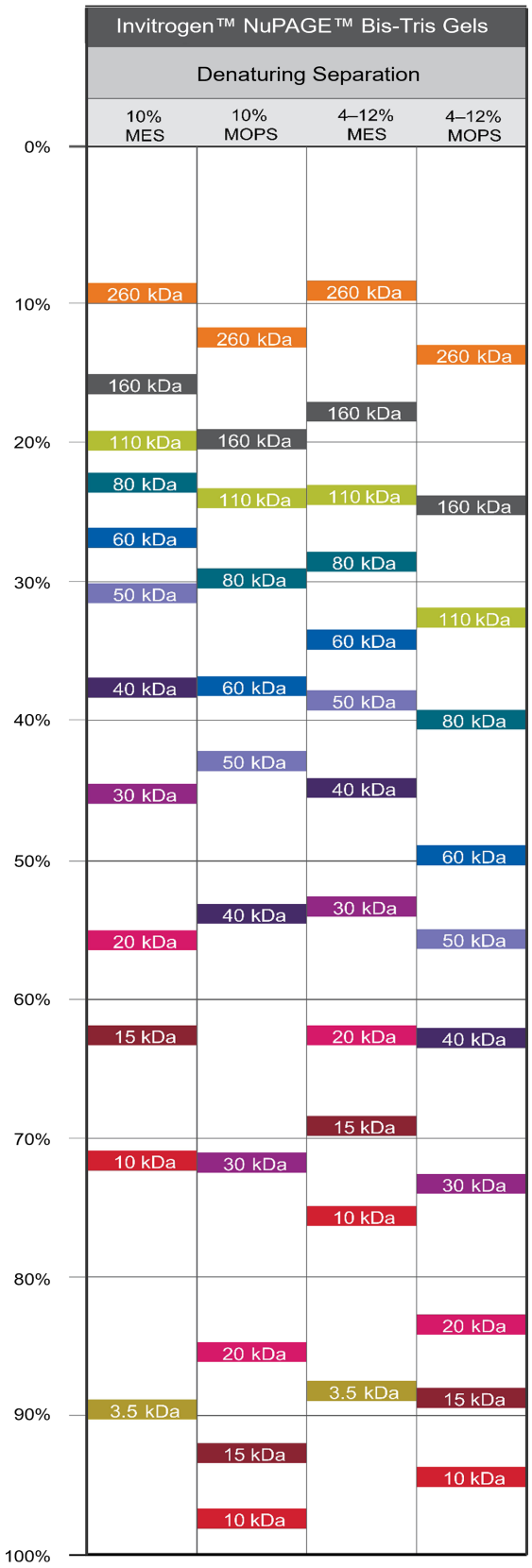
Prepare 1 L of 1X MOPS or MES Dry SDS Running Buffer

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 WedgeWell™ Midi Gels

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



Limited product warranty

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