NuPAGE™ Tris-Acetate Mini Gels

invitrogen

QUICK REFERENCE Pub. No. MAN0009818 Rev. D

Contents and storage

Gel type	Amount	Storage
NuPAGE™ Tris-Acetate Gels	Box of 2 or 10 gels	Store at 2–8°C for up to 8 months. Do not freeze.

Product description

NuPAGE™ Tris-Acetate Gels are precast polyacrylamide gels designed for optimal separation and resolution of large-sized proteins (36–500 kDa) under denaturing or native gel electrophoresis conditions.

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

- **Polyacrylamide percentage**: 7% and 3–8%
- **Well format**: 10, 12, 15, and 2D wells
- **Thickness**: 1.0 mm and 1.5 mm

Required materials

- Protein sample and protein ladder
- 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)

For denaturing applications	For native applications
▪ NuPAGE™ LDS Sample Buffer (4X)	▪ Tris-Glycine Native Sample Buffer (2X)
▪ NuPAGE™ Tris-Acetate SDS Running Buffer (20X)	▪ Novex™ Tris-Glycine Native Running Buffer (10X)

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	
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
2D-well	400 µL	—	12 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	Spectra™ Multicolor High Range Protein Ladder HiMark™ Pre-stained Protein Standard	26625 LC5699
Unstained	NativeMark™ Unstained Protein Standard	LC0725
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™ Tris-Acetate SDS Running Buffer	Resolve large-size proteins under denaturing conditions	LA0041
Tris-Glycine Native Running Buffer	Resolve large-size proteins under native (non-denaturing) conditions	LC2673
NuPAGE™ Transfer Buffer	Wet transfer	NP0006

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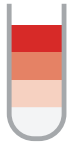
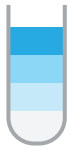
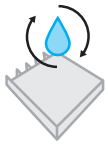
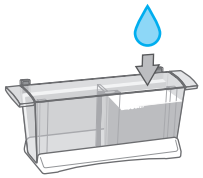
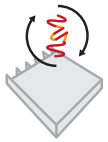
Perform denaturing protein gel electrophoresis using NuPAGE™ Tris-Acetate Mini Gels

<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>Denaturing sample [1]</th><th>Native 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>—</td></tr><tr><td>Tris-Glycine Native Sample Buffer (2X)</td><td>—</td><td>5 µL</td></tr><tr><td>Deionized Water</td><td>to 7.5 µL</td><td>to 5 µL</td></tr><tr><td>Total Volume</td><td>10 µL [2]</td><td>10 µL [2]</td></tr></table>[1] For reduced samples, add NuPAGE™ Reducing Agent (10X) to 1X. [2] See "Choosing a well format" for recommended loading volumes.
Heat denaturing samples at 70°C for 10 minutes. Do not heat native samples.</td></tr>
<tr><td>2</td><td>  </td><td>Prepare buffers</td><td>Denaturing Buffer: Add 50 mL of 20X NuPAGE™ Tris-Acetate SDS Running Buffer to 950 mL of deionized water to prepare 1X SDS Running Buffer.
Native Buffer: Add 100 mL of 10X Tris-Glycine Native Running Buffer to 900 mL of deionized water to prepare 1X Native 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 upper chamber, and 200 mL to the lower chamber (for reduced samples, use running buffer with antioxidant in the lower 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.
For denaturing electrophoresis run for 1 hour at 150 V constant.
For native electrophoresis run for 1.5–3 hours at 150 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 Tris-Acetate SDS Running Buffer

50 mM Tricine, 50 mM Tris Base, 0.1% SDS, pH 8.24

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

Reagent	Amount
Tricine	89.5 g
Tris Base	60.6 g
SDS	10.0 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 Tris-Glycine Native Running Buffer

25 mM Tris Base, 192 mM Glycine, pH 8.3

1. Dissolve the following reagents in 900 mL ultrapure water.

Reagent	Amount
Tris Base	29 g
Glycine	144 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 Transfer Buffer

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

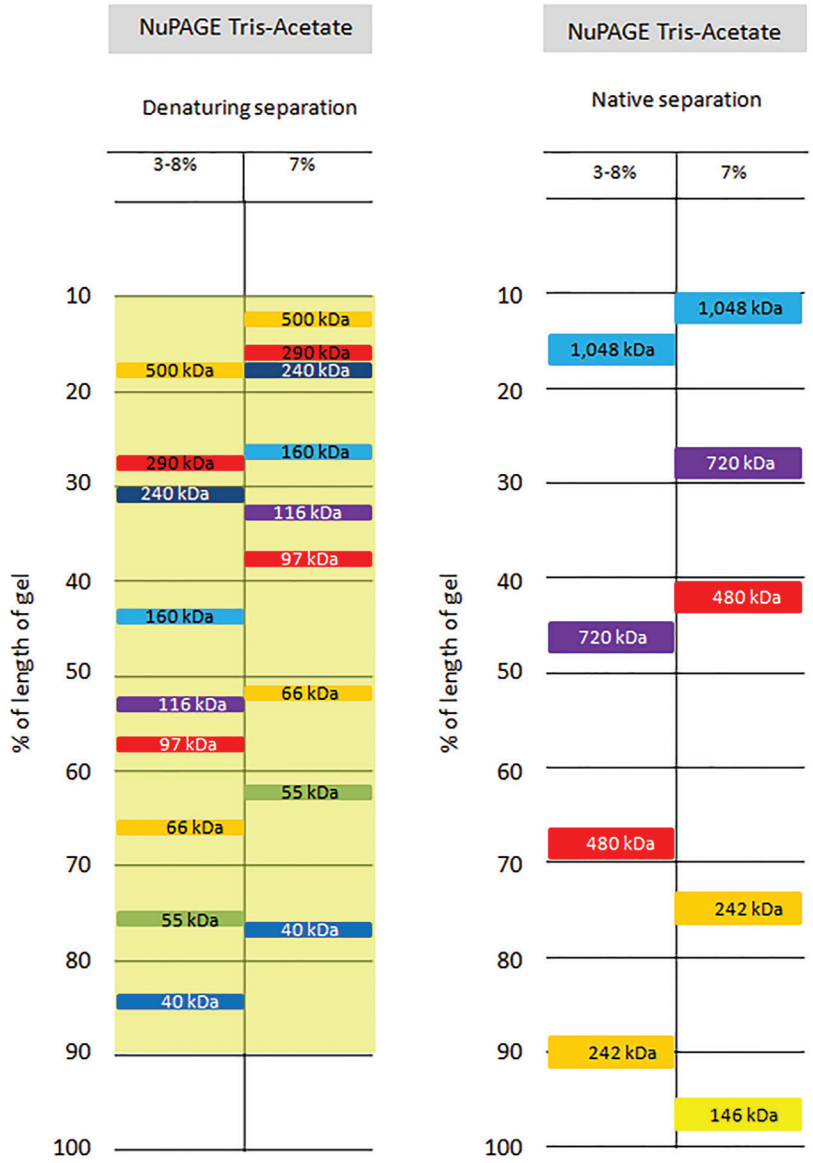
1. Dissolve the following reagents in 100 mL ultrapure water.

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™ Tris-Acetate 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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17 December 2024

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