**QUICK REFERENCE** Pub. No. MAN0007902 Rev. D



# Contents and storage

Gel type	Amount	Storage	
Bolt™ Bis-Tris Plus Gels	Box of 10 gels	Store at 2–8°C for up to 16 months.	
		Do not freeze.	



# **Product description**

Bolt™ Bis-Tris Plus Gels are precast polyacrylamide gels designed for optimal separation and resolution of small- to medium-sized proteins (6-400 kDa) under denaturing gel electrophoresis conditions. The gels feature wedge-shaped WedgeWell™ sample wells with a capacity of up to 60 µL of sample per well.

Bolt<sup>™</sup> Bis-Tris Plus Mini Gels are available with the following specifications:

■ Polyacrylamide percentage: 8%, 10%, 12%, and 4–12%

• Well format: 10, 12, 15, and 17 wells

• Thickness: 1.0 mm



# Required materials

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



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

# Choosing a well format

Well type	Recommended loading volume	Maximum loading volume	Maximum protein load	
10-well	40 µL	60 µL	0.5 μg/band	
12-well	30 µL	45 µL	0.4 μg/band	
15-well	20 μL	35 µL	0.25 µg/band	
17-well	15 µL	30 µL	0.2 μg/band	

### Choosing a protein ladder for your application

Туре	Type Marker	
Pre-Stained	Pre-Stained Protein Ladder PageRuler™ Prestained Protein Ladder PageRuler™ Plus Prestained Protein Ladder	
Unstained	PageRuler™ Unstained Protein Ladder PageRuler™ Unstained Broad Range Protein Ladder	26614 26630
Western blot	iBright <sup>™</sup> Prestained Protein Ladder MagicMark <sup>™</sup> 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.
Bolt™ MOPS SDS Running Buffer	Resolve mid-size proteins	B0001
Bolt™ MES SDS Running Buffer	Resolve small molecular weight proteins	B0002
Bolt™ Transfer Buffer	Wet transfer	BT0006



# Perform denaturing protein gel electrophoresis using Bolt™ Bis-Tris Plus Mini Gels

Step		ep	Action				
			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.				
		Prepare samples	Components	Reduced sample	Non-reduced Sample		
			Sample	xμL	xμL		
1			Bolt™ LDS Sample Buffer (4X)	2.5 μL	2.5 μL		
			Bolt™ Reducing Agent (10X)	1 μL	_		
			Deionized Water	to 6.5 μL	to 7.5 μL		
			Total Volume	10 μL	10 μL		
			Heat samples at 70°C for 10 minutes.				
2		Prepare buffers	Add 50 mL of 20X Bolt™ 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 Bolt™ Antioxidant to 400 mL 1X SDS Running Buffer.				
3		Prepare gel	<ul><li>a. Remove the comb, and rinse the gel wells three times using 1X Running Buffer.</li><li>b. Remove the white tape near the bottom of the gel cassettes.</li><li>c. Place the gels in the mini gel tank.</li></ul>				
4		Load buffers	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).				
5	MIN	Load samples and ladders	a. Load the appropriate volume of your samples in the appropriate wells.      b. Load your protein ladder in the appropriate well.				
			Optimal run times vary depending on gel per	centage and power supply	used for electrophoresis.		
			If using <b>MES</b> Running Buffer, run for 22 minutes at 200 V constant.				
6		Run the gel	If using <b>MOPS</b> Running Buffer, run for 32 minutes at 200 V constant.				
	7		Note: If you are not using a Thermo Fisher So	cientific™ power supply, in:	stall Novex™ Power Supply A	dapters	



#### **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** 

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			Prepare 500 mL of 20X MOPS SDS Running Buffer				
50 mM MES, 50 mM Tris Base, 0.1% SDS, 1 mM EDTA, pH 7.3		50 mM MOPS, 50 mM Tris Base, 0.1% SDS, 1 mM EDTA, pH 7.7					
Dissolve the following reagents in     400 mL ultrapure water.		<ol> <li>Dissolve the following reagents in 400 mL ultrapure water.</li> </ol>					
	Reagent	Amount			Reagent	Amount	
	MES	97.6 g			MOPS	104.6 g	
	Tris Base	60.6 g			Tris Base	60.6 g	
	SDS	10.0 g			SDS	10.0 g	
	EDTA	3.0 g			EDTA	3.0 g	
<ol> <li>Mix well and adjust the volume to 500 mL with ultrapure water.</li> <li>Before electrophoresis, dilute buffer to 1X with water.</li> </ol>		3.	Mix well and ac 500 mL with ul Before electrop to 1X with wate	trapure water. phoresis, dilute			

# Prepare 125 mL of 20X Bis-Tris Transfer Buffer

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

 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 Bolt™ Bis-Tris Plus 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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Life Technologies | Carlsbad, CA 92008 USA | Toll Free in USA 1.800.955.6288

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