

# NuPAGE®

## Frequently Asked Questions (FAQs)

### Frequently Asked Questions

#### What is denaturing DS-PAGE?

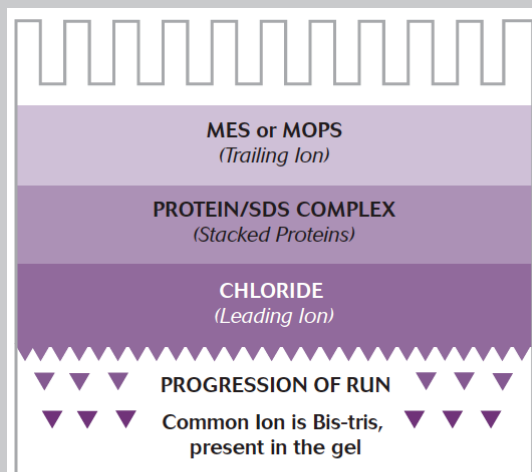
Sodium dodecyl sulfate (SDS) is an anionic detergent that denatures proteins by “wrapping around” the polypeptide backbone. When the protein is heated to 100°C in the presence of excess SDS and thiol reagent, disulfide bonds are cleaved and the protein is fully dissociated into its subunits. Under these conditions, most polypeptides bind SDS at a constant weight ratio (1.4 g of SDS to 1 g of polypeptide). Because the intrinsic charges of the polypeptide are insignificant compared to the negative charges provided by the bound detergent, the SDS-polypeptide complexes have essentially the same negative charge and shape as unbound polypeptides and migrate through the gel strictly according to polypeptide size.

#### What is the chemistry of the NuPAGE® Bis-Tris System?

The NuPAGE® Bis-Tris discontinuous buffer system involves three ions:

- Chloride (Cl<sup>-</sup>) from the gel buffer serves as a leading ion. The gel buffer ions are Bis-Tris (+) and Cl<sup>-</sup> (pH 6.4).
- MES or MOPS (-) serves as the trailing ion in the running buffer. The running buffer ions are Tris (+), MOPS (-)/MES (-), and dodecyl sulfate (-) (pH 7.3–7.7).
- Bis-Tris (+) is the common ion present in the gel, whereas Tris (+) is provided by the running buffer.

The combination of lower-pH gel buffer (pH 6.4) and lower-pH running buffer (pH 7.3–7.7) leads to a significantly lower operating pH of 7 during electrophoresis.



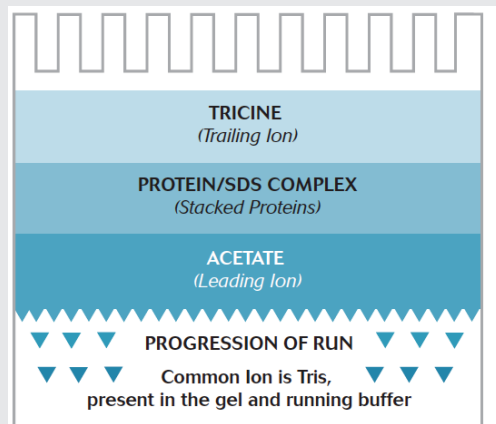
- Gel Buffer Ions are Bis-tris<sup>+</sup>, Cl<sup>-</sup> (pH 6.4)
- Running Buffer Ions are Tris<sup>+</sup>, MES<sup>-</sup> or MOPS<sup>-</sup> and SDS (pH 7.3)
- Gel Operating pH is 7.0

**What is the chemistry of the NuPAGE® Tris-Acetate System?**

The NuPAGE® Tris-acetate discontinuous buffer system involves three ions:

- Acetate (-) from the gel buffer serves as a leading ion. The gel buffer ions are Tris (+) and acetate (-) (pH 7.0).
- Tricine (-) from the running buffer serves as the trailing ion. The running buffer ions are Tris (+), Tricine (-), and dodecyl sulfate(-) (pH 8.3).
- Tris (+) is the common ion present in the gel buffer and running buffer.

The Tris-acetate system also operates at a significantly lower pH of 8.1 during electrophoresis.



- Gel Buffer Ions are Tris<sup>+</sup>, Acetate<sup>-</sup> (pH 7.0)
- Running Buffer Ions are Tris<sup>+</sup>, Tricine<sup>-</sup> and SDS (pH 8.3)
- Gel Operating pH is 8.1

**Why are my sample protein bands running slowly, but the marker bands running normally?**

The sample probably contains a nonionic (or cationic) detergent that is competing with the SDS to coat the proteins. It is the negative charge of the SDS that causes the proteins to migrate to the anode. Samples containing Triton® X-100 will lower the charge density and cause the sample proteins to migrate more slowly. The markers, which come from a separate vial, probably don't contain Triton® X-100 and will therefore migrate correctly. The solution is to wash the sample to remove the nonionic detergent (try using TCA/acetone precipitation).

**Why is my electrophoresis run taking longer than usual?**

If the run is taking longer than usual, the running buffer is too dilute. Make fresh running buffer as described in the user manual. Do not adjust the pH of the 1X running buffer.

**Why is there no current or low current during the electrophoresis run?**

The cause of low or no current is an incomplete circuit during the run. The tape from the bottom of the gel cassette must be removed prior to electrophoresis. In addition, make sure that the buffer covers the sample wells and that the wire connections on the buffer core are intact.

**Why are my proteins streaking after the run?**

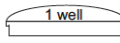
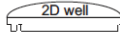
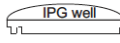
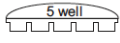
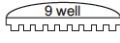
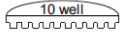
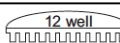

Protein streaking can be caused by high salt concentrations or contaminants such as membranes or DNA in the sample. We recommend that you dialyze the sample to reduce the salt concentration, and centrifuge to remove the contaminants. Another possible reason for streaking is the presence of precipitates in your sample. You can maintain the solubility of your sample by increasing the concentration of SDS.

**Why do I get dumbbell-shaped bands after electrophoresis?**

High protein sample volume can cause incomplete stacking during the run. Load the appropriate volume of sample per well as recommended in question 9. If the sample is too dilute, concentrate the sample using ultrafiltration.

**What is the maximum volume that I can load on my gel?**

The maximum loading volume depends on the well number and thickness of the comb. In addition, the maximum protein load per band is dependent on the detection method. See the recommended loading volumes and protein load per band in the table below.

Well Types	Maximum Load Volume	Maximum Protein Load Per Band by Detection Method		
		Coomassie Staining	Ethidium Bromide	Silver Staining
 1.0 mm	700 µL	12 µg/band	2.4 µg/band	Scale your sample load for the sensitivity of your silver staining kit.  For use with the SilverQuest™ or SilverXpress® Silver Staining Kits, we recommend a protein load of 1 ng/band.
 1.0 mm 1.5 mm	400 µL 600 µL	12 µg/band	2.0 µg/band	
 1.0 mm	7 cm IPG Strip	N/A	N/A	
 1.0 mm	60 µL	2 µg	400 ng/band	
 1.0 mm	28 µL	0.5 µg/band	100 ng/band	
 1.0 mm 1.5 mm	25 µL 37 µL	0.5 µg/band	100 ng/band	
 1.0 mm	20 µL	0.5 µg/band	100 ng/band	
 1.0 mm 1.5 mm	15 µL 25 µL	0.5 µg/band	100 ng/band	

**What causes fuzzy bands after electrophoresis?**

Fuzzy bands are the result of partially denatured or reduced protein samples. Make sure the appropriate amount of DTT or β-mercaptoethanol is added and the sample is heated to 100°C.

**What is the composition of the NuPAGE® buffers?**

Buffer	1X Composition	Storage
NuPAGE® LDS Sample Buffer	Glycerol 10%, Tris base 141 mM, Tris HCl 106 mM, LDS 2%, EDTA 0.51 mM, SERVA® Blue G250 0.22 mM, phenol red_0.175 mM, pH 8.5	4°C
NuPAGE® MES SDS Running Buffer	MES pH 7.2 50 mM, Tris base 50 mM, SDS 0.1%, EDTA 1 mM, pH 7.3	4°C
NuPAGE® MOPS SDS Running Buffer	MOPS 50 mM, Tris base 50 mM, SDS 0.1%, EDTA 1 mM, pH 7.7	4°C
NuPAGE® Transfer Buffer	Bicine 25 mM, Bis-Tris (free base) 25 mM, EDTA 1.0 mM, chlorobutanol 0.05 mM, pH 7.2	4°C
NuPAGE® Tris-Acetate SDS Running Buffer	Tris base 50 mM, Tricine 50 mM, SDS 0.1%, pH 8.24	4°C

What happens if an incorrect buffer system is used on a NuPAGE® Bis-Tris Gel?

If you used the...	Instead of the....	Then....
<u>NuPAGE® MES SDS Running Buffer</u>	<u>NuPAGE® MOPS SDS Running Buffer</u>	<u>the</u> run time of the gel is decreased by ~10–15 minutes. <u>there</u> is decreased separation and resolution for proteins >36 <u>kDa</u> .
<u>NuPAGE® MOPS SDS Running Buffer</u>	<u>NuPAGE® MES SDS Running Buffer</u>	<u>the</u> run time of the gel is increased by ~10–15 minutes. <u>the</u> lower molecular weight proteins (<14 <u>kDa</u> ), which are normally well resolved, are not resolved while the high molecular weight proteins are resolved more than normal.
<u>Novex® Tris-Glycine SDS Sample Buffer</u>	<u>NuPAGE® LDS Sample Buffer</u>	<u>some</u> bands are not very sharp and there is increased protein fragmentation.
<u>Novex® Tricine SDS Sample Buffer</u>	<u>NuPAGE® LDS Sample Buffer</u>	<u>the</u> band sharpness is not affected, but the lanes will be slightly wider due to the increased amount of SDS and buffer salts from the <u>Tricine</u> Sample Buffer.
<u>Novex® Tris-Glycine SDS Running Buffer and the Novex® Tris-Glycine SDS Sample Buffer</u>	<u>NuPAGE® MOPS or MES SDS Running Buffer and the NuPAGE® LDS Sample Buffer</u>	<u>the</u> gel will have an extremely long run time of 3–4 hours due to the low migration of the glycine ions at neutral <u>pH</u> . <u>the</u> sensitivity of the staining for high molecular weight proteins is decreased. <u>the</u> bands are more compressed at the bottom of the gel, regardless of the gel percentage and the bands have a cupped appearance at the bottom of the band.
<u>Novex® Tricine SDS Running Buffer and the Tricine SDS Sample Buffer</u>	<u>NuPAGE® MOPS or MES SDS Running Buffer and the NuPAGE® LDS Sample Buffer</u>	<u>the</u> run time of the gel is increased by 1–2 hours due to the slow migration of the <u>tricine</u> ions at neutral <u>pH</u> . <u>there</u> may be background streaking in the lanes.

What are the electrophoresis conditions for NuPAGE® gels?

Gel Type	Voltage for Electrophoresis	Expected Current (Gel)	Gel Run Time
NuPAGE® Bis-Tris SDS-PAGE (denaturing, non-reducing)	200 V constant	100–125 mA (start), 60–70 mA (end)	35–50 minutes
NuPAGE® Bis-Tris SDS-PAGE (denaturing, reducing)	200 V constant	110–125 mA (start), 70–80 mA (end)	35–50 minutes
NuPAGE® Tris-Acetate SDS-PAGE (denaturing, nonreducing)	150 V constant	40–55 mA (start), 25–40 mA (end)	60 minutes
NuPAGE® Tris-Acetate SDS-PAGE (denaturing, reducing)	150 V constant	40–55 mA (start), 25–40 mA (end)	60 minutes
NuPAGE® Tris-Acetate SDS-PAGE (native)	20–150 V constant	40–55 mA (start), 25–40 mA (end)	1–12 hours

**What are the separation ranges for NuPAGE® gels?**

<b>Gel</b>	<b>Min Resolution</b>	<b>Max Resolution</b>	<b>Denaturing</b>	<b>Sample Buffer</b>	<b>Running Buffer</b>	<b>Transfer Buffer</b>
NuPAGE® 10% Bis-Tris (With MES)	2.5 kDa	200 kDa	1	NuPAGE® LDS Sample Buffer	NuPAGE® MES SDS Running Buffer	NuPAGE® Transfer Buffer
NuPAGE® 10% Bis-Tris (With MOPS)	14 kDa	220 kDa	1	NuPAGE® LDS Sample Buffer	NuPAGE® MOPS SDS Running Buffer	NuPAGE® Transfer Buffer
NuPAGE® 12% Bis-Tris (With MES)	2 kDa	200 kDa	1	NuPAGE® LDS Sample Buffer	NuPAGE® MES SDS Running Buffer	NuPAGE® Transfer Buffer
NuPAGE® 12% Bis-Tris (With MOPS)	6 kDa	200 kDa	1	NuPAGE® LDS Sample Buffer	NuPAGE® MOPS SDS Running Buffer	NuPAGE® Transfer Buffer
NuPAGE® 3–8% Tris-Acetate	31 kDa	400 kDa	1	NuPAGE® LDS Sample Buffer	NuPAGE® Tris-Acetate SDS Running Buffer	NuPAGE® Transfer Buffer
NuPAGE® 3–8% Tris-Acetate (Native)	NA	NA	0	Tris-Glycine Native Sample Buffer	Tris-Glycine Native Running Buffer	Tris-Glycine Transfer Buffer
NuPAGE® 4–12% Bis-Tris (With MES)	2.5 kDa	200 kDa	1	NuPAGE® LDS Sample Buffer	NuPAGE® MES SDS Running Buffer	NuPAGE® Transfer Buffer
NuPAGE® 4–12% Bis-Tris (With MOPS)	12 kDa	220 kDa	1	NuPAGE® LDS Sample Buffer	NuPAGE® MOPS SDS Running Buffer	NuPAGE® Transfer Buffer
NuPAGE® 7% Tris-Acetate (Native)	NA	NA	0	Tris-Glycine Native Sample Buffer	Tris-Glycine Native Running Buffer	Tris-Glycine Transfer Buffer
NuPAGE® 7% Tris-Acetate	31 kDa	400 kDa	1	NuPAGE® LDS Sample Buffer	NuPAGE® Tris-Acetate SDS Running Buffer	NuPAGE® Transfer Buffer

What happens if an incorrect buffer system is used on a NuPAGE® Tris-Acetate Gel?

Sample Buffer	Running Buffer	Antioxidant	Results
Novex® Tris-Glycine SDS	NuPAGE® Tris-Acetate SDS	Yes	Fuzzy, smeared bands.
Novex® Tricine SDS	NuPAGE® Tris-Acetate SDS	Yes	Bands are not very sharp.
NuPAGE® LDS	NuPAGE® MES SDS or NuPAGE® MOPS SDS	Yes	Bands are diffuse and have a "U" shape. More low molecular weight proteins are visible.
NuPAGE® LDS	Novex® Tris-Glycine SDS	No	The run time is twice as long as the Tris-Acetate Buffer system. The band resolution is poor.
NuPAGE® LDS	Novex® Tricine SDS	No	The run time is 10–15 minutes faster than the Tris-Acetate Buffer system. Reduced protein bands are diffuse while non-reduced large molecular weight protein bands are smeared.
Novex® Tris-Glycine SDS	Novex® Tris-Glycine SDS	No	The run time is much longer than the Tris-Acetate Buffer system and the bands are very faint with a streaked background. Fewer low molecular weight bands are resolved.
Novex® Tricine SDS	Novex® Tricine SDS	No	The run time is 10–15 minutes faster than the Tris-Acetate Buffer system and reduced protein bands are not sharp. The overall performance is acceptable.

If I am currently using a 10% Tris-Glycine or Tricine gel, which NuPAGE® gel do you recommend?

Use the following table to determine the appropriate NuPAGE® Bis-Tris or Tris-acetate gel for a similar Tris-glycine or Tricine gel.

Currently using	Recommended NuPAGE® gel
4% Tris-glycine	NuPAGE® 3–8% Tris-Acetate (+TA buffer)
6% Tris-glycine	NuPAGE® 3–8% Tris-Acetate (+TA buffer)
10% Tris-glycine	NuPAGE® 8% Bis-Tris (+MOPS buffer) NuPAGE® 10% Bis-Tris (+MOPS buffer)
12% Tris-glycine	NuPAGE® 10% Bis-Tris (+MOPS buffer)
14% Tris-glycine	NuPAGE® 12% Bis-Tris (+MOPS buffer)
16% Tris-glycine	NuPAGE® 12% Bis-Tris (+MES buffer)
18% Tris-glycine	NuPAGE® 12% Bis-Tris (+MES buffer)
4–12% Tris-glycine	NuPAGE® 3–8% Tris-Acetate (+TA buffer) NuPAGE® 4–12% Bis-Tris (+MOPS buffer)
4–20% Tris-glycine	NuPAGE® 4–12% Bis-Tris (+MES buffer)
8–16% Tris-glycine	NuPAGE® 4–12% Bis-Tris (+MOPS buffer)
10–20% Tris-glycine	NuPAGE® 12% Bis-Tris (+MOPS buffer)
10% Tricine	NuPAGE® 8% Bis-Tris (+MES buffer) NuPAGE® 10% Bis-Tris (+MES buffer)
16% Tricine	NuPAGE® 4–12% Bis-Tris (+MES buffer) NuPAGE® 12% Bis-Tris (+MES buffer)
10–20% Tricine	NuPAGE® 4–12% Bis-Tris (+MES buffer)

What is the shelf life of NuPAGE® gels?

Novex® NuPAGE® Bis-Tris Gels have a shelf life of 12 months when stored at 4–25°C. Novex® NuPAGE® Tris-Acetate Gels have a shelf life of 8 months when stored at 4°C.

What are the storage conditions for NuPAGE® gels?

Store Novex® NuPAGE® Bis-Tris gels at 4–25°C and Novex® NuPAGE® Tris-Acetate Gels at 4°C. Do not freeze NuPAGE® gels. Using expired gels or improperly stored gels may result in poor band resolution.

What sizes are the NuPAGE® mini and midi gels?

The NuPAGE® mini gel is 8 x 8 cm and the NuPAGE® midi gel is 8.7 x 13.3 cm.

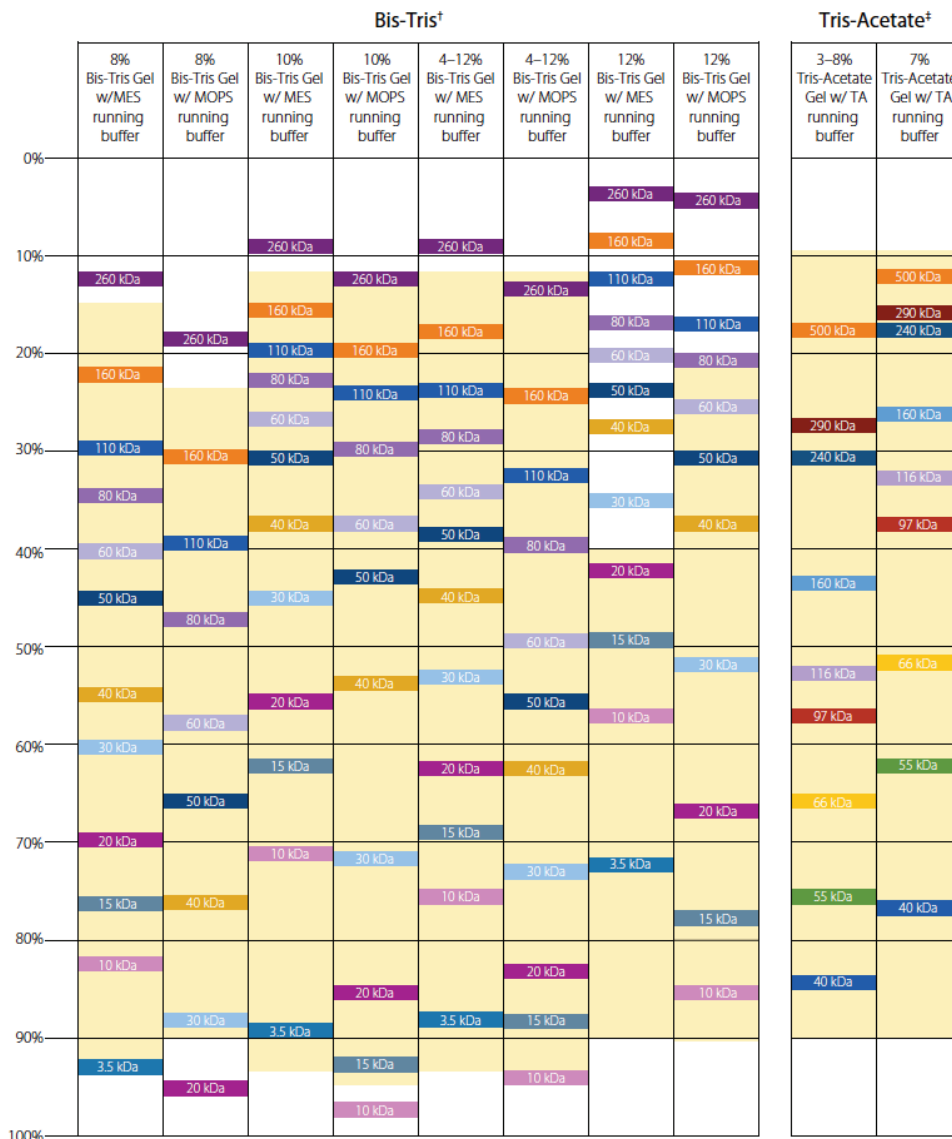
Why is NuPAGE® LDS sample buffer (pH 8.5) better than a traditional Laemmli-style sample buffer?

NuPAGE® LDS sample buffer (pH 8.5) maintains a >7.0 pH environment when heated to 70°C. This is ideal not only for protein reduction and alkylation, but also for preserving protein integrity. The pH of traditional Laemmli-style sample buffer changes from 6.8 to 5.2 when heated to 100°C. This lower pH is known to induce aspartyl-prolyl (Asp-Pro) peptide bond cleavage, which leads to protein degradation.

Why do proteins transfer better from a NuPAGE® gel?

NuPAGE® gels are able to separate proteins using lower acrylamide concentrations than those required for Tris-glycine gels. This more open gel matrix allows more efficient transfer of proteins onto membranes during western blotting. In addition, the NuPAGE® transfer buffer maintains neutral pH and prevents reoxidation of reduced samples during protein transfer onto a membrane. This avoids sample modifications that can occur at the alkaline pH of the traditional transfer buffers and maintains sample antigenicity.

What are the migration patterns for NuPAGE® gels?



† Migration patterns of Novex® Sharp Protein Standards (Cat. no. LC5800, Pre-stained; Cat. no. LC5801, Unstained) on NuPAGE® Novex® Bis-Tris Gels

‡ Migration patterns of HiMark™ Unstained Standard (Cat. no. LC5688) on NuPAGE® Novex® Tris-Acetate Gels

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