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Product Information

TruPAGE™ Precast Gel System

TECHNICAL BULLETIN

Product Description

TruPAGE™ gels offer improved resolution, tear resistance, and an extended shelf-life over the currently available precast polyacrylamide gels. TruPAGE gels are formulated with Triethanolamine (TEA)-Tricine, featuring a neutral pH running environment.

TruPAGE gel cassettes have a hassle-free design.

- Extra tall wells to prevent lane-to-lane overflow.
- No comb to remove, preventing torn or bent wells. Instead, the cassettes lock well fingers in place.
- No tape to remove

Components of TruPAGE Precast Gel System

See Appendix for reagent formulations.

TruPAGE Precast Gels are available in 10 × 10 cm and 10 × 8 cm formats, which makes them compatible with the most popular electrophoresis equipment from most manufacturers such as Sigma-Aldrich, Bio-Rad®, Thermo Fisher®, C.B.S. Scientific, Hoefer®, and others.

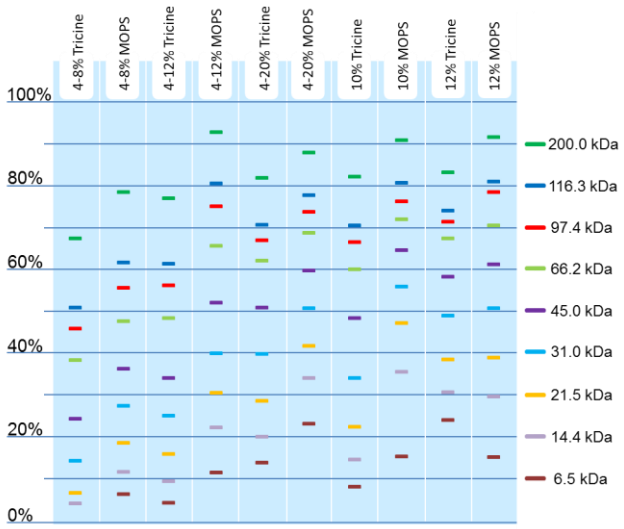
TruPAGE LDS Sample Buffer (Catalog Number PCG3009) should **ALWAYS** be used to prepare protein samples for use with TruPAGE precast gels to achieve optimal band resolution and sharpness. The sample buffer is specifically formulated to complement the TruPAGE running buffer system and to deliver the optimal denaturing conditions without causing sample degradation. Effective protein denaturation occurs at 70 °C instead of the much higher temperature required with Laemmli buffer.

TruPAGE DTT Sample Reducer (Catalog Number PCG3005) reduces disulfide bonds in protein samples. It is convenient and easy to use when performing SDS-PAGE under reducing conditions.

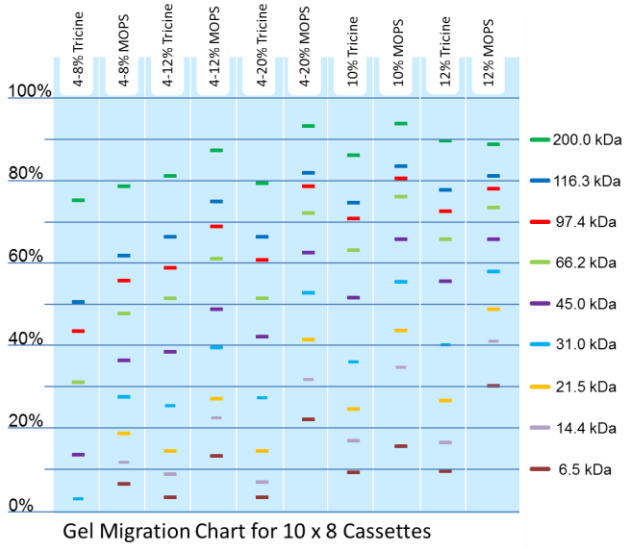
TruPAGE Running Buffers are available in two formulations, TEA-Tricine SDS (Catalog Number PCG3001) and Tris-MOPS SDS Express (Catalog Number PCG3003). **TruPAGE Running Buffers must be used with TruPAGE precast gels** in order to provide effective protein resolution. Refer to the migration charts (see Figure 1) to determine which gel-buffer system is best suited for the application. **Tris-MOPS SDS Express running buffer features a shorter run time; however, the inner chamber must be supplemented with TruPAGE Antioxidant.**

Note: TEA-Tricine SDS running buffer should be used if resolving proteins on 4–8% gels, which is ideal for larger molecular weight proteins.

Figure 1.
Migration Charts



Gel Migration Chart for 10 x 10 Cassettes



Gel Migration Chart for 10 x 8 Cassettes

TruPAGE Running Antioxidant (Catalog Number PCG3007) is added to the running buffer of the cathode (inner) chamber to prevent protein sample reoxidation during electrophoresis and is required when performing reducing SDS-PAGE with Tris-MOPS SDS Express Running Buffer (PCG3003).

TruPAGE Transfer Buffer (Catalog Number PCG3011) is a Tris-Glycine based blotting buffer optimized for transferring proteins from TruPAGE precast gels to PVDF or nitrocellulose membranes.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage and Stability

TruPAGE gels feature an extended shelf-life of up to 24 months from the date of manufacture when stored at 2–8 °C.

Procedures

Running Buffer Preparation

TruPAGE TEA-Tricine SDS and Tris-MOPS SDS Express Running Buffers are supplied as 20× concentrate solutions. For best results, it is recommended to prepare fresh 1× buffer for every run. To prepare 1 L of 1× running buffer, simply dilute 50 mL of the 20× running buffer concentrate with 950 mL of ultrapure water. If performing reducing SDS-PAGE with Tris-MOPS SDS Express Running Buffer, supplement the cathode (inner) chamber buffer with TruPAGE 800× Running Antioxidant (250 µL per 200 mL of running buffer) to prevent sample reoxidation. The use of Running Antioxidant is not required with TEA-Tricine SDS Running Buffer due to the mild reducing nature of TEA.

Sample Preparation and Gel Loading

For optimal results only use TruPAGE formulated buffers and reagents when preparing and running samples with TruPAGE gels.

1. Samples should be prepared just prior to electrophoresis (see Table 1).

Table 1.
Preparation of Electrophoresis Samples

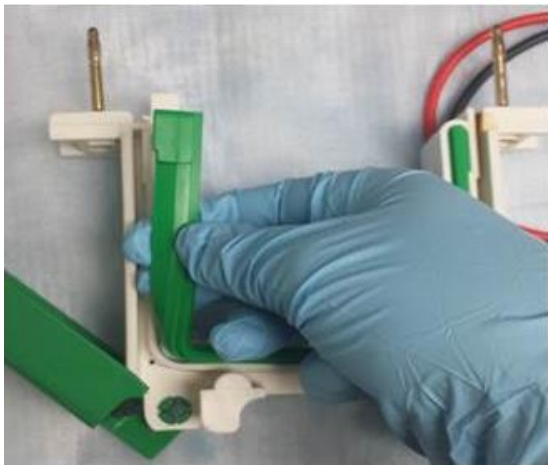
Reagent	**Reduced Sample** (µL)	**Non-reduced Sample** (µL)
Protein Sample	x	x
4× Sample Buffer	2.5	2.5
10× Sample Reducer*	1	–
Ultrapure Water	6.5–x	7.5–x
Total Volume	10	10

* DDT or β-mercaptoethanol (BME) can be used as a reducing agent. If so, add DTT to a final concentration of 25 mM or add BME to a final concentration of 10%. Note: Do not store reduced samples for >2 hours as they may reoxidize.

2. Heat samples for 10 minutes at 70 °C. Do **not** boil samples.
3. Rinse the gel wells with ultrapure water prior to sample loading. TruPAGE wells **MUST** be rinsed prior to loading samples for removal of trace storage buffer to achieve optimal band resolution and sharpness.

4. Insert gel into the gel running tank.
   Note: When using Bio-Rad tanks, reverse the device gasket to the flat side to ensure proper seal (see Figure 2).

Figure 2.
Flipping the Bio-Rad gasket



When using Bio-Rad running tanks, remember to reverse the green device gasket to its flat (back) side. If this is not done, the protruding portion of the gasket will prevent the TruPAGE gel from making a tight seal and the inner chamber buffer will leak into the outer chamber.

5. Fill the cathode chamber with 1× running buffer and ensure a proper seal prior to filling the anode chamber.
   Note: When using Tris-MOPS SDS Express running buffer while performing reducing SDS-PAGE, supplement the cathode (inner) chamber running buffer with TruPAGE 800× Running Antioxidant (250 µL/200 mL of running buffer). The use of the antioxidant is not required when running non-reduced protein samples.
6. Load the samples into the wells. Do not exceed well capacity when loading samples, 35 µL for 12 well gels and 20 µL for 17 well gels.
   Note: Use thin gel-loading tips for better settling of the samples towards the bottom of the wells.

Running the Gel
Once the samples are loaded and buffer chambers are filled, run the gel at constant voltage (180 V) until the blue dye front reaches the bottom of the gel cassette. Depending on the gel percentage and running buffer, run times can range from 30–70 minutes. The Tris-MOPS SDS Express Running Buffer typically produces run times shorter (~10 minutes) than those of TEA-Tricine SDS Running Buffer when used with equivalent gels. Expected current during the gel run should remain below 110 mA/gel (maximum: 20 W) and will decrease gradually as the run progresses.

Gel Staining
TruPAGE Precast Gels are compatible with popular gel staining protocols.

A. Coomassie Brilliant Blue R-250
Prepare Coomassie Brilliant Blue R-250 stain solution [40% ethanol/10% acetic acid/0.1% (w/v) Brilliant Blue R-250 (Catalog Number B0149)]. Volumes in procedures are for 1 gel.

1. Heated Procedure (50–70 °C)
Do not heat the gel to ABOVE 70 °C, as the stain solution may get too hot and dissolve the gel matrix. Heat solutions separately in a microwave oven prior to incubation with the gel. Microwave ovens vary; always check solution temperature and adjust heating times.
   a. Run gels as normal and remove the gel from the cassette.
   b. In a clean staining dish rinse the gel with ultrapure water 3 times for 5 minutes each time in order to remove SDS, which could cause high background staining.
   c. Heat 50 mL of stain solution for 30 seconds on high (600 W). Place the gel in the warm Coomassie Blue R-250 stain solution and incubate 15–30 minutes with gentle agitation.
   d. Warm 50 mL of destain solution (20% ethanol/5% acetic acid) for 30 seconds on high (600 W). Remove gel from staining solution. Destain for 1–24 hours.
      Note: Destained gels may be stored up to 2 days in 100 mL of destain solution supplemented with 20 mL of 20% (w/v) NaCl in water.

2. Ambient Temperature Procedure
   a. Run gels as normal and remove the gel from the cassette.
   b. In a clean staining dish rinse the gel with ultrapure water 3 times for 5 minutes each time in order to remove SDS, which could cause high background staining.
   c. Place the gel in 50 mL of Coomassie Blue R-250 stain solution for 1–2 hours and incubate with gentle agitation.
   d. Remove gel from staining solution. Destain with 50 mL of destain solution (20% ethanol/ 5% acetic acid) for 3–24 hours.
   Note: Destained gels may be stored up to 2 days in 100 mL of destain solution supplemented with 20 mL of 20% (w/v) NaCl in water.

B. Coomassie Brilliant Blue G-250

Prepare Coomassie Brilliant Blue G-250 stain solution [Dissolve 60–80 mg of Brilliant Blue G-250 (Catalog Number 27815) per liter of ultrapure water and mix for 2–4 hours. Add 3 mL of concentrated HCl and mix]. Volumes in procedures are for 1 gel.

1. Heated Procedure (50–70 °C)

Do not heat the gel to temperatures ABOVE 70 °C, as the stain solution may get too hot and dissolve the gel matrix. Heat solutions separately prior to incubation with the gel. Microwave ovens vary; always check solution temperature and adjust heating times.

   a. Run gels as normal and remove the gel from the cassette.
   b. Heat 100 mL of ultrapure water for 1 minute on high (600 W). Wash the gel with warm ultrapure water for 5 minutes. Repeat warm water wash twice.
   c. Heat 25 mL of stain solution for 20 seconds on high (600 W). Place the gel in warm stain solution and incubate 30 minutes with gentle agitation.
   d. Warm 100 mL of destain solution (10% ethanol) for 30 seconds on high (600 W). Remove gel from stain solution and destain for 15 minutes to 16 hours.

   Note: Destained gels may be stored up to 2 days in 100 mL of destain solution supplemented with 20 mL of 20% (w/v) NaCl in water.

C. EZBlue™ Gel Staining Reagent (Catalog Number G1041)
   1. Run gels as normal and remove the gel from the cassette.
   2. In a clean staining dish rinse the gel with ultrapure water 3 times for 5 minutes each time in order to remove SDS, which could cause high background staining.
   3. TruPAGE gels do not need to be fixed when using EZBlue Stain. Place the gel in 20–40 mL of EZBlue stain and incubate at room temperature for at least 1 hour with gentle agitation. Staining overnight will not increase the background.
   4. Destain the gel with ultrapure water until the background becomes clear, residual SDS in the gel may cause the background to appear slightly blue. Change the water often to enhance the contrast between the stained protein and the background. Full background destaining should take only about 1–2 hours at room temperature.
   Note: Staining can be achieved faster if the temperature is increased.

D. Instant*Blue*™ Ultrafast Protein Stain (Catalog Number ISB1L)
   1. Run gels as normal and remove the gel from the cassette.
   2. Place the gel directly into 20–30 mL of Instant*Blue* stain solution and incubate at room temperature with gentle agitation. Protein bands will begin developing immediately and a suitable intensity can be achieved in as little as 15 minutes.
   3. Gels can be kept in staining solution and imaged as soon as the desired staining intensity has been achieved. Alternatively, if stained for at least 1 hour, the gel can be imaged and stored in ultrapure water.

E. Silver-Stain Solutions
1. Run gels as normal and remove the gel from the cassette.
2. In a clean staining dish, rinse the gel with ultrapure water 3 times for 5 minutes each time in order to remove SDS from the gel, which could cause high background staining.
3. TruPAGE gels need to be fixed when using silver staining solutions. Fix the proteins for 10 minutes with a 50 mL/gel fixing solution of (50% methanol/10% acetic acid/20 mM sodium bisulfite). For the sodium bisulfite, one can use TruPAGE Antioxidant (Product Number PCG3007): 250 µL per 50 mL fixative.
Note: Substitute this fixing step with the manufacturer's silver staining protocol.
4. After the fixing step, follow the remainder of the manufacturer's silver stain protocol.

F. SYPRO® Ruby Protein Gel Stain (Catalog Number S4942)
1. Run gels as normal and remove the gel from the cassette.
2. Place gel in fixing solution (50% ethanol/ 10% acetic acid). Incubate the gel in fixing solution for 30 minutes with gentle agitation.
3. Remove fixing solution and rinse gel with ultrapure water.
4. Pour 50 mL of stain into a clean, detergent-free polypropylene or polyvinyl chloride staining dish. Do not use glass dishes. Cover from light and incubate for 3–24 hours with gentle agitation.
5. Transfer gel to a clean staining dish and wash with ultrapure water. To achieve higher contrast, the gels can be destained with destaining solution (10% methanol/7% acetic acid) for 1 hour with gentle agitation.
6. Remove destaining solution and rinse the gel with ultrapure water.

Gel Blotting

Follow the general guidelines for the blotting apparatus.

A. Wet Transfer
1. Equilibrate TruPAGE gels in 1× Transfer Buffer when using most electroblotting apparatuses, including Trans-blot® from Bio-Rad and XCell Surelock® tanks from Thermo Fisher. However, if using the Dual Run system from Sigma-Aldrich, equilibrate TruPAGE gels in 2× Transfer Buffer. See Table 2 for preparation of 1× Transfer Buffers or Table 4 for 2× Transfer Buffer from TruPAGE Transfer Buffer.

Table 2.
Preparation of 1× Transfer Buffer

	Membrane	
Reagent	**Nitrocellulose**	**PVDF**
Transfer Buffer (mL)	50	50
Methanol (mL)	200	100
Ultrapure Water (mL)	750	850
Total Volume (mL)	1,000	1,000

2. Equilibrate membrane (PVDF or nitrocellulose) in freshly prepared, chilled 1× Transfer Buffer for 5–10 minutes prior to blotting.
Note: If using PVDF, activate precut membrane with 100% methanol according to manufacturer's instructions prior to equilibrating in Transfer Buffer. Do not expose nitrocellulose membranes to concentrated organic solvents.
3. Assemble the electroblotting transfer sandwich according to the manufacturer's instructions. See Table 3 for the recommended wet transfer conditions using the Trans-Blot Cell, XCell SureLock, and Dual Run and Blot modules. **This information is provided for reference only; please review the blotting module manufacturer's documentation for proper operating instructions.**

Table 3.
Wet Transfer Conditions

Wet Transfer Conditions	**Trans-Blot**	**XCell SureLock**	**Dual Run and Blot**
Voltage (V)	50-100	35	200
Blot Time (hours)	1–2	1–1.5	1.5–2
Expected Current (mA)	250	200	180–220

B. Semi-Dry Transfer

1. Equilibrate TruPAGE gels in 2× Transfer Buffer. See Table 4 for preparation of 2× Transfer Buffer from TruPAGE Transfer Buffer.

Table 4.
Preparation of 2× Transfer Buffer for Semi-Dry Transfer

	Membrane	
Reagent	**Nitrocellulose**	**PVDF**
Transfer Buffer (mL)	100	100
Methanol (mL)	200	100
Ultrapure Water (mL)	700	800
Total Volume (mL)	1,000	1,000

2. Equilibrate membrane (PVDF or nitrocellulose) in freshly prepared, chilled 2× Transfer Buffer for 5–10 minutes prior to blotting.
   Note: If using PVDF, activate precut membrane with 100% methanol according to manufacturer instructions prior to equilibrating in Transfer Buffer. Do not expose nitrocellulose membranes to concentrated organic solvents.
3. Assemble the electroblotting transfer sandwich according to the manufacturer's instructions. See Table 5 for the recommended semi-dry transfer conditions **This information is provided for reference only; please review the blotting module manufacturer's documentation for proper operating instructions.**

Table 5.
Semi-Dry Transfer Conditions

Semi-Dry Transfer Conditions	
Voltage (V)	25
Blot Time (hours)	0.5–1
Expected Current (mA)	250–300

C. Dry Transfer

iBLOT® Conditions

1. Equilibrate TruPAGE gels and membrane in ultrapure water.
   Note: If using PVDF, activate precut membrane with 100% methanol according to manufacturer instructions prior to equilibrating in ultrapure water. Do not expose nitrocellulose membranes to concentrated organic solvents.
2. Assemble the electroblotting transfer device according to manufacturer's instructions. Recommended conditions: use manufacturer's program P3 (20 V, 7 minutes, 13 minutes MAX).

Bio-Rad Trans-Blot® Turbo™ Conditions

Assemble the electroblotting transfer device according to manufacturer's instructions. Recommended conditions: use manufacturer's program High MW (>150 kDa) for 1 mini-gel (10 minutes, 1.3 A constant, up to 25 V).

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Appendix

Reagent Formulations with suggested products.

Sigma-Aldrich recommends using high purity buffer reagents to ensure proper conductivity properties of the running buffers for optimal protein resolution.

TruPAGE Buffers are specifically formulated for and MUST be used with TruPAGE precast gels. TruPAGE Buffers are non-proprietary and can be made by researchers. For best results, use buffers made in the last 6 months.

Pre-mixed TruPAGE solutions are formulated with proprietary stabilizers added to extend shelf life.

20× TruPAGE TEA-Tricine SDS Running Buffer
(Catalog Number PCG3001)

1.2 M Triethanolamine (Catalog Number 90279)
0.8 M Tricine (Catalog Number T5816)
2.0% (w/v) Sodium Dodecyl Sulfate (SDS) (Catalog Number L3771)

Triethanolamine	179.0 g
Tricine	143.3 g
SDS	20 g
Ultrapure Water to Total Volume	1.0 L

The pH should be between 8.2–8.3 at 25 °C.

20× TruPAGE Tris-MOPS SDS Express Running Buffer
(Catalog Number PCG3003)

1.2 M Trizma® (Catalog Number RD008)
0.6 M MOPS (Catalog Number RDD003)
2.0% (w/v) Sodium Dodecyl Sulfate (Catalog Number L3771)

Trizma	145.2 g
MOPS	125.6 g
SDS	20.0 g
Ultrapure Water to Total Volume	1.0 L

The pH should be between 8.2–8.3 at 25 °C.

Note: Antioxidant (5 mM sodium bisulfite) is required in the inner chamber if using this buffer.

800× TruPAGE Running Antioxidant
(Catalog Number PCG3007)

4 M Sodium Bisulfite (Catalog Number 243973)

Sodium Bisulfite	4.2 g
Ultrapure Water to Total Volume	10 mL

4× TruPAGE LDS Sample Buffer
(Catalog Number PCG3009)

40% (w/v) Glycerol (Catalog Number G5516)
4% (w/v) Lithium Dodecyl Sulfate (LDS) (Catalog Number L9781)
4% Ficoll® 400 (Catalog Number F2637)
0.025% Phenol Red (Catalog Number P3532)
0.025% Brilliant Blue G250 (Catalog Number 27815)
2 mM EDTA (Catalog Number E6758)

Glycerol	4.0 g
LDS	0.40 g
Triethanolamine*	1.2 g
Ficoll® 400	0.40 g
Phenol Red	2.5 mg
Brilliant Blue G250	2.5 mg
EDTA	7.0 mg
Ultrapure Water to Total Volume	10 mL

The final pH should be between 7.7–7.8 at 25 °C.
* 0.8 M Triethanolamine solution, pH 7.6 should be made prior to addition of other reagents. Adjust the pH with HCl.

10× TruPAGE DTT Sample Reducer
(Catalog Number PCG3005)

0.25 M Dithiothreitol (Catalog Number D9163)

DTT	38.6 mg
Ultrapure Water to Total Volume	1.0 mL

20× TruPAGE Transfer Buffer
(Catalog Number PCG3011)

0.25 M Trizma base (Catalog Number RDD008)
1.92 M Glycine (Catalog Number G8898)

Trizma	30.3 g
Glycine	144.1 g
Ultrapure Water to Total Volume	1.0 L

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Inner (Cathode) chamber buffer leaking.	The gel is not properly seated in the chamber.	Ensure the gel cassette is seated in the chamber according to the gel running equipment manufacturer's instructions.
	10 × 8 cm gel is being used with equipment designed for 10 × 10 cm gels.	10 × 8 cm gel cassettes are 2.5 mm thinner than 10 × 10 cm cassettes and cannot be used with gel boxes designed for 10 × 10 cm gels. Always use gels compatible with equipment available. Equipment compatibility information is available on TruPAGE gel product detail pages and in this TruPAGE Technical Bulletin.
	Bio-Rad running box gasket was not flipped to the flat side.	Flip the device gasket to its flat backside. Instructions can be found in this TruPAGE Technical Bulletin.
Gel does not fit into the running box chamber.	10 × 10 cm gel is being used with equipment designed for 10 × 8 cm gels.	Only use 10 × 8 cm and 10 × 10 cm gels with gel running equipment designed for gels of these formats. Equipment compatibility information is available on TruPAGE gel product detail pages and in this TruPAGE Technical Bulletin.
No current during run.	Buffer leaking from the inner chamber.	See Problem, “Inner (Cathode) chamber buffer leaking” for resolution.
	Low voltage output from the power supply.	Ensure that running voltage is set according to instructions found in this TruPAGE Technical Bulletin. Ensure that the power supply is functioning properly.
	Running buffer was not properly diluted.	Make fresh 1× TruPAGE Running Buffer for wet transfer and 2× TruPAGE Running Buffer for semi-dry transfer from 20× concentrated solution.
Gels overheating during electrophoresis.	Recommended run voltage exceeded.	Follow TruPAGE gel running instructions found in this TruPAGE Technical Bulletin.
	Outer (anode) buffer chamber is not filled properly.	Ensure to fill the outer chamber, as the buffer in the outer chamber serves as coolant during gel run.
	TruPAGE gels are being run alongside gels from other manufacturers.	Gels with differing gel chemistry have different electrical resistivity properties. Do not run gels from different manufacturers alongside each other in the same gel running box.
Dye front changes color (i.e., yellow).	Under filled outer (anode) buffer chamber is causing buffer starvation.	Add more 1× TruPAGE Running Buffer to the outer buffer chamber.
Misshaped protein bands and/or crooked lanes.	Gel overheating during run.	See Problem “Gels overheating during electrophoresis” for resolution.
	Incompatible running buffer used.	Only run TruPAGE gels using TruPAGE formulated running and sample loading buffers. Buffer compositions can be found in this TruPAGE Technical Bulletin.

Troubleshooting Guide (continued)

Problem	Possible Cause	Suggested Solution
Excessive streaking of protein bands.	Incompatible sample buffer used.	Always prepare samples with TruPAGE LDS Sample Buffer. Buffer composition can be found in this TruPAGE Technical Bulletin.
	Too much protein loaded into the wells.	Prevent sample overload by limiting the amount of protein loaded per well. Maximum recommended protein loading: 10 μg for purified protein, 100 μg for lysate.
	Precipitate/particulates in the sample.	Centrifuge the sample to remove any insoluble matter or decrease total sample concentration to ensure complete solubility of proteins.
	Salt concentration is too high.	If sample lysis/storage buffer contains a high salt concentration, it may be necessary to perform dialysis prior to starting sample preparation for electrophoresis.
Protein samples diffusing rapidly throughout the gel.	Gel has been frozen.	Always store TruPAGE gels at recommended storage conditions (2–8 °C).
Prestained protein markers indicate different apparent molecular masses.	Prestained protein markers used have not been calibrated for use with TruPAGE gels.	Dyes used for staining protein markers behave differently in different buffer systems, which will affect the migration patterns of prestained proteins. Calibrate prestained protein markers against unstained proteins of known size or use ColorBurst™ Electrophoresis Marker (Catalog Number C1992), a solution of precalibrated, prestained protein markers.