INSTRUCTIONS

Precise Tris-Glycine Gels

25245-25274

2435.2

Thermo Scientific™ Precise™ Tris-Glycine Gels, 10cm × 10cm, Selection Table

Product Number	Percent Acrylamide	Running Buffer	Cassette Size (W×H×T)*	Wells	Well Volume	Separation Range (kDa)
25245	8	Tris-Glycine-SDS	10 × 10 × 0.7cm	10	50µL	205-45
25246	10	Tris-Glycine-SDS		10	50µL	205-24
25247	12	Tris-Glycine-SDS		10	50µL	205-14
25248	8-16	Tris-Glycine-SDS		10	50µL	205-14
25249	4-20	Tris-Glycine-SDS		10	50µL	205-6.5
25250	8	Tris-Glycine-SDS	10 × 10 × 0.7cm	12	30µL	205-45
25251	10	Tris-Glycine-SDS		12	30µL	205-24
25252	12	Tris-Glycine-SDS		12	30µL	205-14
25253	8-16	Tris-Glycine-SDS		12	30µL	205-14
25254	4-20	Tris-Glycine-SDS		12	30µL	205-6.5

*W = width, H = height, T = thickness

Thermo Scientific Precise Tris-Glycine Gels, 10cm × 8cm, Selection Table

Product Number	Percent Acrylamide	Running Buffer	Cassette Size (W×H×T)*	Wells	Well Volume	Separation Range (kDa)
25260	8	Tris-Glycine-SDS	10 × 8 × 0.5cm	10	50µL	205-45
25261	10	Tris-Glycine-SDS		10	50µL	205-24
25262	12	Tris-Glycine-SDS		10	50µL	205-14
25263	8-16	Tris-Glycine-SDS		10	50µL	205-14
25264	4-20	Tris-Glycine-SDS		10	50µL	205-6.5
25265	8	Tris-Glycine-SDS	10 × 8 × 0.5cm	12	30µL	205-45
25266	10	Tris-Glycine-SDS		12	30µL	205-24
25267	12	Tris-Glycine-SDS		12	30µL	205-14
25268	8-16	Tris-Glycine-SDS		12	30µL	205-14
25269	4-20	Tris-Glycine-SDS		12	30µL	205-6.5
25270	8	Tris-Glycine-SDS	10 × 8 × 0.5cm	15	25µL	205-45
25271	10	Tris-Glycine-SDS		15	25µL	205-24
25272	12	Tris-Glycine-SDS		15	25µL	205-14
25273	8-16	Tris-Glycine-SDS		15	25µL	205-14
25274	4-20	Tris-Glycine-SDS		15	25µL	205-6.5

*W = width, H = height, T = thickness

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Introduction

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) is used to determine the approximate molecular weights of proteins with reference to the mobility of standard proteins on the same gel. Gradient gels provide the appropriate gel pore size making molecular weight estimation more accurate by sharpening stained protein bands (Figure 1).

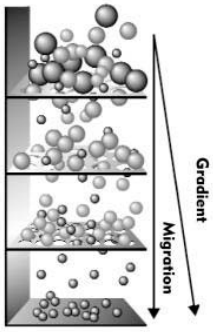


Figure 1. Gradient gel electrophoresis.

The most popular buffer system for SDS-PAGE is the Laemmli system [Nature 227:680-686 (1970)]. This system is normally limited by extended running times and gel instability from hydrolysis of polyacrylamide to acrylic acid in alkaline conditions; however, the Precise Tris-Glycine Gels can withstand the chemical changes that alter gel conductivity and the gel migration pattern because of their formulation.

When the gel pH is neutral, hydrolysis does not occur. Precise Tris-Glycine Gels are cast at pH 7, yielding a long shelf life and assured reproducibility of the migration pattern (Figure 2). The advantages of Precise Tris-Glycine Gels are as follows:

- Sample wells reinforced with plastic eliminate damage when loading
- Sample well dividers do not deform or fall over
- Easy-to-open cassette
- Universal cassette design
- Up to 12-month shelf-life warranty from date of purchase
- 40-60-minute run time
- 90-minute (wet transfer) or 30-minute (semi-dry) transfer time

Please visit our website for a complete list of electrophoresis-related reagents, including molecular weight markers, gel stains and products for Western blotting and sample preparation.

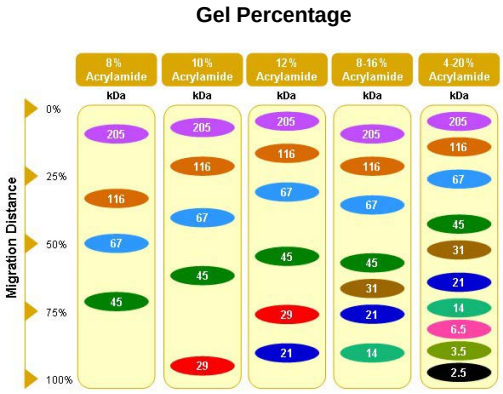


Figure 2. Thermo Scientific Precise Tris-Glycine Gel migration table.

Precise Tris-Glycine Gels Specifications

Cassette Dimensions:	10cm × 8cm × 0.5cm or 10cm × 10cm × 0.7cm
Gel Dimensions:	8cm × 6.8cm × 0.1cm or 8cm × 8.8cm × 0.1cm
Storage Conditions:	4°C
Shelf Life:	Up to 12 months from date of purchase
Stacking Gel:	4%
Buffer System in Gel:	Tris-HCl, pH 7
SDS:	None
Required Running Buffer:	Tris-Glycine-SDS
Recommended Sample Buffer:	Tris-HCl-SDS or LDS-SDS

Compatible Gel Tanks

- Novex™ XCell I and II™
- Novex XCell II Surelock™
- Bio-Rad™ Mini-PROTEAN™ II and 3
- Bio-Rad Mini-PROTEAN Tetra Cell
- Hoefer™ Tall Mighty Small™ (SE 280)
- Hoefer Mighty Small II (SE 260/SE 250)
- IBI Universal Protein System
- Owl Road Runner, Penguin

Instructions for using Precise Tris-Glycine Gels

A. Preparing the Gel Cassette and Gel Tank

Note: Please see the note at the end of this section (Section A) concerning special instructions for using the Bio-Rad Mini PROTEAN Cell with Precise Tris-Glycine 10×8cm gels. There are no special instructions for using the Novex XCell unit with Precise Tris-Glycine 10×10cm gels.

1. Dissolve one packet of Thermo Scientific BupH Tris-Glycine-SDS Running Buffer (Product No. 28378) in 500mL of ultrapure water, or dilute 10X Tris-Glycine-SDS 10-fold in water. This buffer volume of running buffer (500mL) is sufficient for some electrophoresis units but others will require larger volumes. (See Buffer Recipes on page 6 for preparing a 10X stock of the required Tris-Glycine-SDS Running Buffer.)
2. Remove a Precise Tris-Glycine Gel from the pouch and insert the gel into the gel running apparatus (refer to the apparatus manufacturer's instructions).
3. Add sufficient volume of Tris-Glycine-SDS running buffer into the inner tank of the gel running apparatus to cover the sample wells by 5-7mm.
4. Add the remaining volume of Tris-Glycine-SDS running buffer to the outer tank to ensure proper cooling. The buffer in the outer tank should be approximately level with the bottom of the sample wells.

   Note: For best resolution, the buffer in the outer tank must reach the bottom of the sample wells to keep the gels cool.
5. Using a transfer (Pasteur) pipette, rinse the sample wells thoroughly with Tris-Glycine-SDS running buffer to remove air bubbles and to displace any storage buffer. The gel may be pre-electrophoresed for 5-10 minutes.

   Note: To use a Bio-Rad Mini-PROTEAN Cell apparatus, remove the gasket from the inner frame (Figure 3), turn it around so the flat side is facing outwards and re-insert into the inner frame.

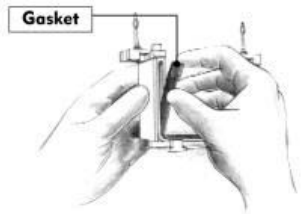


Figure 3. Removing the gasket from the inner frame of the Bio-Rad Mini-PROTEAN Cell.

B. SDS Sample Preparation

Add one part LDS Sample Buffer, Non-Reducing (4X) (Product No. 84788) to three parts sample. Alternatively, use the sample buffer recipe on page 6.

For lyophilized samples, mix 100µL of Sample Buffer (1X) per milligram of protein. Heat sample for 3-5 minutes at approximately 100°C. Clarify by centrifugation at ~1000 × *g* for 3 minutes and collect the supernatant.

Note: If the sample is thermally labile, leave at room temperature for 1 hour with occasional mixing. Dissolution may be helped by sonication. If breakage of disulfide bonds is required (reducing conditions), add 10mM dithiothreitol (DTT) or 5mM β-mercaptoethanol (BME) final concentration.

C. Sample Loading

Note: These Precise Protein Gels are designed to open without any mechanical devices (e.g., keys, knives, etc.). However, this easy-to-open design requires that you verify the center pin at the top of the cassette is pinched tight to prevent leakage during sample loading and electrophoresis.

Apply 5-50µg (total protein) per sample well. Each sample well holds from 25-50µL depending on the well capacity. For a sample with a total protein concentration of 10mg/mL, apply 2-5µL per well. For best results, use pipette tips specifically designed for gel loading.

Caution: Inserting the pipette tip too far into the cassette may cause the cassette to separate. For best results, use only gel-loading tips.

Note: Optimal sample size must be established empirically. Overloading the gel will cause smearing and distortion. Excessive loading of proteins with free carbohydrate may also result in band distortion or failure of the protein to penetrate into the gel (See Troubleshooting Section).

D. Running Conditions

Connect the gel rig leads to the power supply and electrophorese according to Table 1.

Table 1. Electrophoresis conditions for Thermo Scientific Precise Tris-Glycine Gels.

	Approximate Current		
Voltage	**Start**	**Finish**	**Run Time per Gel***
185V	80-100mA/gel	20-40mA/gel	~40 minutes (10 × 8cm gels)
185V	65-85mA/gel	30-50mA/gel	~60 minutes (10 × 10cm gels)

*Gel running time is dependent on the gel gradient and the temperature in the laboratory. These run times are recommended at a laboratory temperature of 20°C.

E. Removing a Gel from the Cassette

1. Once the run is finished, remove the gel from the gel tank according to the tank manufacturer's instructions.
2. To open the cassette, pull the sides apart or insert a coin in one of the slots on the side and twist (Figure 4a).
3. Pull the top plate of the cassette away from the bottom plate (Figure 4b). The two halves will snap apart completely, exposing the gel.
4. Loosen the gel at the bottom with water and remove.

Figure 4a and 4b. Opening the cassette to expose the gel.

Staining and Drying Gels

All standard SDS staining procedures may be used with Precise Tris-Glycine Gels.

Gels can be dried using standard drying techniques. When using commercially available gel drying reagents, follow the manufacturer's instructions.

Coomassie Staining

- Before staining, wash the gel three times for 5 minutes each in 200mL of water. The wash step will remove SDS from the gel, decrease the required staining time and increase staining sensitivity.
- Commercially available stains, such as Thermo Scientific™ Imperial™ Protein Stain (Product No. 24615) or Thermo Scientific™ GelCode™ Blue Stain (Product No. 24590), as well as homemade coomassie stains may be used. Best results are obtained with methanol concentrations of < 30%.

Silver Staining

- For best results, before staining, wash gels in ultrapure water for 10-15 minutes to remove SDS.
- Commercially available stains, such as Thermo Scientific™ Pierce™ Silver Stain Kit (Product No. 24612) or Pierce Color Silver Stain Kit (Product No. 24597), as well as homemade silver stains may be used.

Western Blotting Protocols for Precise Tris-Glycine Gels

Standard blotting procedures may be used with Precise Tris-Glycine Gels. Below are protocols for wet and semi-dry blotting.

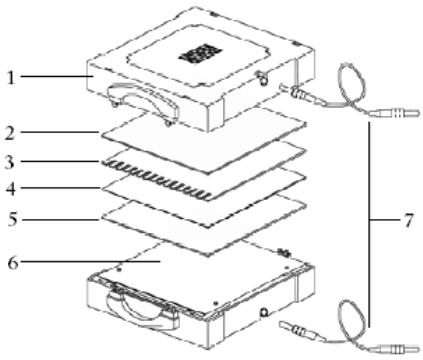
Wet Blotting Protocol

1. Cool the transfer buffer to 4°C.
2. Equilibrate gels in the transfer buffer for 5 minutes.
3. Soak filter papers in the transfer buffer.
4. Soak membrane(s) in transfer buffer (PVDF membranes must be wetted in 100% methanol first prior to equilibration in the transfer buffer).
5. Soak the foam pad in transfer buffer.

6. Assemble the transfer sandwich as follows:
    - Cathode (– – –)
    - Foam Pad
    - Filter paper
    - Gel
    - Transfer Membrane
    - Filter paper
    - Foam Pad
    - Anode (+++)
7. Pour the transfer buffer through the sandwich and place it into the apparatus. Fill the apparatus with transfer buffer.
8. Transfer at 40V for 120 minutes or 200mA for 90 minutes (maintain buffer temperature at ~4°C).
9. Gently remove gel from sandwich and rinse with transfer buffer.
10. Use a cotton swab to remove any adhering gel from the membrane.

Semi-Dry Blotting Protocol

1. Cool the transfer buffer to 4°C.
2. Soak the filter paper, membrane and gel in Tris-Glycine Transfer Buffer (Product No. 28380) for 15 minutes.
3. Assemble the blotting sandwich in a semi-dry blotting apparatus according to the instructions:



1. Upper lid (cathode, – – –)
2. Filter paper
3. Gel
4. Membrane
5. Filter paper
6. Lower base (anode, + + +)
7. Electrical cables

4. Transfer the blot for 30 minutes at 20V (or 45min at 15V).
5. Remove the gel from the sandwich and rinse with transfer buffer.

Staining Membranes

The Pierce Reversible Protein Stain Kits (Product No. 24580 for nitrocellulose; Product No. 24585 for PVDF) contain Reversible Protein Stain, Destain and Stain Eraser. Sensitivity is ~25ng of protein (~10 times the sensitivity of Ponceau S stain) and staining and destaining can be accomplished in 15 minutes.

Buffer Recipes

Use high-purity reagents and high-purity water when making buffers.

Tris-Glycine-SDS Running Buffer (10X)	
Tris Base	29g
Glycine	144g
SDS	10g
Add ultrapure water to 1L	

- Before use, dilute 10-fold with water.

Protein Transfer Buffer
Dissolve one BupH™ Tris-Glycine Transfer Buffer Pack (Product No. 28380) in 400mL of ultrapure water
Add 100mL methanol (20%) and cool to 4°C

Note: The pH of the buffer should be ~8.0

Note: Addition of 0.05% SDS will improve the transfer of proteins out of the gel onto PVDF membrane. SDS reduces the ability for proteins to bind to nitrocellulose membranes.

Sample Loading Buffer (2X)	
10% (w/v) Sodium Dodecyl Sulfate (SDS) Electrophoresis Grade	4.0mL
Glycerol	2.0mL
0.1% (w/v) Bromophenol Blue	1.0mL
0.5M Tris-HCl, pH 6.8	2.5mL
2-β-Mercaptoethanol or DTT*	2-5% v/v
Add ultrapure water to 10mL	

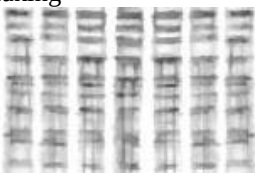
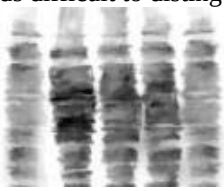
*Add if cleavage of disulfide bonds is desired.

Alternative Protein Transfer Buffer	
Tris Base	3.00g
Bicine	4.08g
Ethanol or Methanol	100mL
Add ultrapure water to 1L	

Note: The pH of the buffer should be ~8.0

Note: Addition of 0.05% SDS will improve the transfer of proteins out of the gel onto PVDF membrane. SDS reduces the ability for proteins to bind to nitrocellulose membranes.

Troubleshooting

Problem	Cause	Solution
Distorted protein bands 	Air bubbles were in the sample wells, between the gel and cassette, or at the bottom of the cassette	Use a transfer pipette to displace the air bubbles from the sample wells
	Sample contained appreciable carbohydrate	Remove the carbohydrate by enzymatic or chemical means
	Sample contained lipoproteins	Use a gel with a large pore size at the top or try adding a nonionic detergent
Streaking 	Poorly soluble or weakly charged particles (such as carbohydrates) were in the sample	Centrifuge samples
		Change pH of sample buffer
		Heat sample in the presence of SDS
Bands difficult to distinguish 	Incorrect gel selection, sample overloading and insufficient cooling buffer	Select a gel that separates in the desired molecular weight range
		Reduce sample size
		Increase buffer volume in the outer tank
		For proteins of similar molecular weight, a 2D separation may be required

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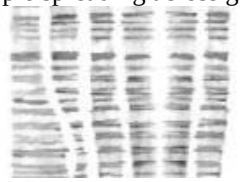
Sample spreading across gel 	Excess salt was in the sample	Reduce salt by dialysis or ultra-filtration
	Too much protein was applied to the gel	Optimize the amount of protein applied to the gel
Protein denaturation and band inversion	Excessive heating	Start with chilled buffer (< 15°C)
Diffuse protein zones in the gel after staining	SDS was present in the gel	Wash gel extensively (3 × 5 minutes) with ultrapure water and use 30% methanol to destain gel
	Protein bands were diffusing	Use 10% TCA to fix the proteins in the gel

Related Thermo Scientific Products

26616 **PageRuler™ Prestained Protein Ladder, 10 to 170kDa**

26619 **PageRuler Plus Prestained Protein Ladder, 10 to 250kDa**

26634 **Spectra™ Multicolor Broad Range Protein Ladder, 10 to 260kDa**

39000 **Lane Marker Reducing Sample Buffer (5X),** 5mL

77720 **Bond-Breaker™ TCEP (Odorless reducing agent),** 5mL

28378 **BupH Tris-Glycine-SDS Running Buffer,** 40 packs

28362 **10X Tris-Glycine Transfer Buffer,** 1L

28380 **BupH Tris-Glycine Transfer Buffer,** 40 packs

24615 **Imperial™ Protein Stain,** 1L

24620 **PageBlue™ Protein Staining Solution,** 1L

24594 **GelCode™ Blue Safe Protein Stain,** 1L

24590 **GelCode Blue Stain Reagent,** 500mL

24612 **Pierce Silver Stain Kit**

24597 **Pierce Color Silver Stain Kit**

24582 **E-Zinc™ Reversible Stain Kit**

Please see our website for more information on our complete line of Western blotting products, including:

- Nitrocellulose, PVDF and nylon transfer membranes
- Blocking buffers
- Labeled secondary antibodies
- X-ray film
- Chemiluminescent, chemifluorescent and colorimetric substrates
- Wash buffers and detergents
- Background Eliminator for film
- Western blot stripping buffer

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