INSTRUCTIONS

Thermo SCIENTIFIC

Precise™ Protein Gels

25200-25244

1472.5

Precise Protein Gel Selection Table*

Product Number	% Acrylamide	Running Buffer	Cassette Size	Wells	Well Volume	Separation Range (kDa)
25200	8	Tris-HEPES-SDS	100 × 85	10	50 µl	205-45
25201	10	Tris-HEPES-SDS	× 4.5 mm	10	50 µl	205-24
25202	12	Tris-HEPES-SDS		10	50 µl	205-14
25203	8-16	Tris-HEPES-SDS		10	50 µl	205-14
25204	4-20	Tris-HEPES-SDS		10	50 µl	205-6.5
25220	8	Tris-HEPES-SDS	100 × 85	12	30 µl	205-45
25221	10	Tris-HEPES-SDS	× 4.5 mm	12	30 µl	205-24
25222	12	Tris-HEPES-SDS		12	30 µl	205-14
25223	8-16	Tris-HEPES-SDS		12	30 µl	205-14
25224	4-20	Tris-HEPES-SDS		12	30 µl	205-6.5
25240	8	Tris-HEPES-SDS	100 × 85	15	25 µl	205-45
25241	10	Tris-HEPES-SDS	× 4.5 mm	15	25 µl	205-24
25242	12	Tris-HEPES-SDS		15	25 µl	205-14
25243	8-16	Tris-HEPES-SDS		15	25 µl	205-14
25244	4-20	Tris-HEPES-SDS		15	25 µl	205-6.5

*Choose a Precise Protein Gel equivalent to the gel that is used in the Laemmli system.

Important Note:

Do not use Tris-glycine-SDS running buffer with Precise Protein Gels.
Use only Tris-HEPES-SDS running buffer as described in these instructions.

Table of Contents

Pierce Biotechnology 3747 N. Meridian Road | PO Box 117 Rockford, IL 61105 USA | (815) 968-0747 (815) 968-7316 fax | www.thermo.com/pierce

Introduction

Within the last 20 years, electrophoresis has rapidly evolved from a high-resolution method of relatively limited application into a widely used analytical technique of unrivalled resolving power and exceptional versatility. The most commonly used electrophoresis technique is SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis). SDS solubilizes proteins by coating them with a uniform negative charge, disrupting the majority of non-covalent bonds and reducing their hydrophobicity. SDS treatment largely removes differences between proteins in both charge and conformation, making size the main determinant of electrophoretic mobility. This mobility 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).

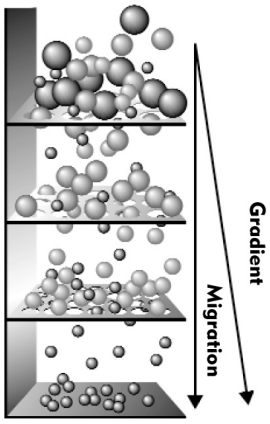


Figure 1. Gradient gel electrophoresis.

The most popular buffer system for SDS-PAGE is the Laemmli system [Nature 227, 680–686 (1970)]. This system, however, is limited by extended running times and gel instability from hydrolysis of polyacrylamide to acrylic acid in alkaline conditions. This chemical change alters gel conductivity, causing a constant change in the gel's migration pattern with time.

When the gel pH is neutral, hydrolysis does not occur. Precise Protein Gels are cast at pH 7, yielding a long shelf life and assured reproducibility of the migration pattern (Figure 2). The extra stability of Precise Protein Gels is combined with a new Tris-HEPES running buffer to give speed and excellent resolution with the same size ranges as the Laemmli system. Advantages of Precise Protein Gels are as follows:

- Sample wells reinforced with plastic eliminate damage when loading
- Sample well dividers do not deform or fall over
- Sample wells hold up to twice the volume of other gels
- Easy-to-open cassette
- Universally compatible cassette design
- 12-month shelf-life warranty
- 45-minute run time
- 90-minute transfer time

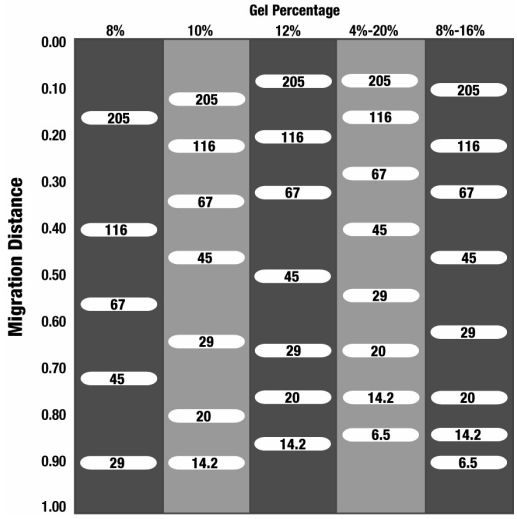


Figure 2. Protein migration table of the various gels.

Please visit our web site or catalog for a complete list of electrophoresis reagents including MW markers, gel staining, Western blotting and products for sample preparation.

Precise™ Protein Gels Specifications

Cassette Dimensions:	10 cm × 8.5 cm × 4.5 mm
Gel Dimensions:	8 cm × 5.8 cm × 1 mm
Storage Conditions:	4°C
Shelf Life:	12 months from date of purchase
Stacking Gel:	4%
Buffer System in Gel:	Tris-HCl, pH 7
SDS:	None
Required Running Buffer:	Tris-HEPES-SDS
Recommended Sample Buffer:	Tris-HCl-SDS

Compatible Gel Tanks

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

Instructions for using Precise Protein Gels

A. Preparing the Gel Cassette and Gel Tank

Important: Please see notes at the end of this section (Section A) concerning special instructions for using Bio-Rad® Mini PROTEAN Cell and NOVEX Tanks.

1. Dissolve one packet of BupH™ Tris-HEPES-SDS Running Buffer (Product No. 28398) in 500 ml of ultrapure water. This buffer volume of running buffer (500 ml) is sufficient for one electrophoresis unit. (See Buffer Recipes on page 6 for instructions for preparing a 10X stock of the required Tris-HEPES-SDS Running Buffer.)

   Note: Do not use Tris-glycine-SDS running buffer. This buffer formulation is not compatible with Precise Gels. Proteins will not migrate or resolve properly. Use only the required Tris-HEPES-SDS running buffer.

2. Remove Precise Protein Gel from the pouch and insert into the gel running apparatus. Refer to the apparatus manufacturer's instructions.
3. Add sufficient volume of Tris-HEPES-SDS running buffer into the inner tank of the gel running apparatus to cover the sample wells by 5-7 mm.
4. Add the remaining volume of Tris-HEPES-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-HEPES-SDS running buffer to remove air bubbles and to displace any storage buffer. The gel may be pre-electrophoresed for 5-10 minutes.

Notes for using Bio-Rad Mini-PROTEAN Cell and NOVEX Tanks:

Using a Bio-Rad Mini-PROTEAN Cell

- 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.

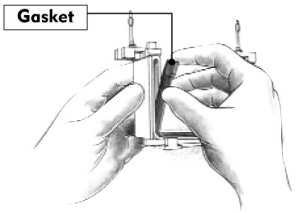


Figure 3. Removing the gasket from the cell.

Using a NOVEX Gel Running Apparatus

Note: A tight seal must be formed between the gel cassette and the gasket of the running frame to prevent leakage. In the NOVEX Tanks, adaptor plates must be used to form a tight seal. Below are instructions for adaptor plate placements when running only one gel and when running two gels.

Precise Protein Gels are 8.5 cm high and are positioned lower in the Novex Tank than other brands of precast gels. However, the resolving portion of the gel is the same length as a Novex Gel.

Running One Precise Protein Gel in a NOVEX Tank

- Two adaptor plates are required when running just one gel.
- Place the gel onto the running frame.
- Place two adaptor plates onto the back of the running frame. (Ensure that the wells are facing away from the inner tank so that buffer does not leak.)
- Put the running frame into the tank.
- Using the wedge device, clamp the running frame and the gels into place (Figure 4). The running frame should be fixed tightly and the gels should not be able to move.

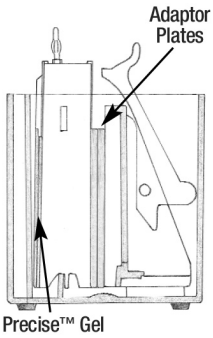


Figure 4. Adaptor plates placement when using one gel.

Running Two Precise Protein Gels in a NOVEX Tank

- One adaptor plate is required when running two gels.
- Place both gels onto the running frame.
- Place one adaptor plate behind the back gel. (Ensure that the cassettes are facing away from the inner tank so that the buffer does not leak.)
- Place the running frame into the tank.
- Using the wedge device, clamp the running frame and the gels into place (Figure 5). The running frame should be fixed tightly and the gels should not be able to move.

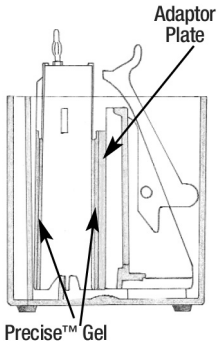


Figure 5. Adaptor plate placement when using two gels.

B. SDS Sample Preparation

Add 1 part Lane Marker Reducing or Non-Reducing 5X Sample Buffer (Product No. 39000 or 39001) to 4 parts sample. Alternatively, use the sample buffer recipe on page 6.

For solubilizing lyophilized samples, mix 100 µl of Tris-HCl SDS Sample Buffer (1X) per mg of protein. Heat sample for 3-5 minutes at approximately 100°C. Clarify by centrifugation at 6,000 rpm for 3 minutes and collect the supernatant.

Note: Tris-HEPES gels are compatible with Tris-Glycine SDS sample loading buffer (non-reducing and reducing). If the sample is thermally labile, add SDS to the sample as a 4% solution. Incubate sample for 1 hour at room temperature then add an equal volume of solution containing 0.05 M HEPES, 1 M sodium chloride, 10% glycerol and 0.05% Bromophenol blue. Dissolution may be helped by sonication.

C. Sample Loading

Apply 5-50 µg (total protein) per sample well. Each sample well holds from 25-50 µl. For a sample with a total protein concentration of 10 mg/ml, apply 2-5 µl 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.

Note: Optimal sample size must be established empirically. Overloading 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 (Figure 6) and electrophorese according to Table 1.

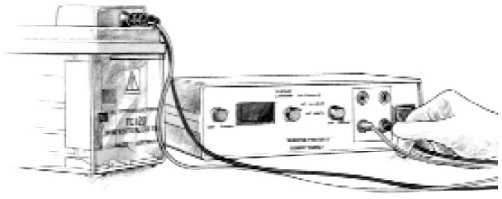


Figure 6. Gel tank and power supply.

Table 1. Electrophoresis conditions for Precise Gels.

	Approximate Current		
Voltage	Start	Finish	Run Time per Gel*
100-120 V	100-130 mA/gel	40-60 mA/gel	~45 minutes

*Gel running time is dependent on 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 manufacturer's instructions.
2. To open the cassette, insert a coin in one of the slots on the side and twist (Figure 7a).
3. Pull the top plate of the cassette away from the bottom plate (Figure 7b). The two halves will snap apart completely, exposing the gel.
4. Loosen the gel at the bottom with water and remove.

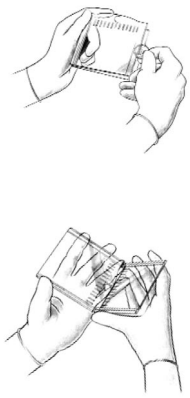


Figure 7a and 7b. Opening the cassette to expose the gel.

Staining and Drying Gels

All standard SDS staining procedures may be used with Precise Protein Gels.

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

Coomassie Staining

- To increase staining sensitivity and decrease staining time, wash gel three times for 5 minutes each in 200 ml of water before staining. This wash will remove SDS from the gel.
- Commercially available stains, such as Imperial™ Protein Stain (Product No. 24615) and GelCode™ Blue Stain (No. 24590), and 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 Silver Stain Kit II (Product No. 24612) and Color Silver Stain Kit (Product No. 24597), and homemade silver stains may be used.

Western Blotting Protocols for Precise Protein Gels

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

Wet Blotting Protocol

1. Cool the transfer buffer to 4°C.
2. Equilibrate the gels in transfer buffer for 5 minutes.
3. Soak filter papers (8 × 10 cm) in transfer buffer.
4. Soak membrane(s) (8 × 10 cm) in transfer buffer (PVDF membranes must be wetted in methanol first and then equilibrated in aqueous solution).
5. Soak the Scotch-Brite™ Pads in transfer buffer.
6. Assemble the transfer sandwich as follows:

    • Cathode (– – –)
    • Scotch-Brite Pad
    • 2x filter paper
    • Gel
    • Transfer Membrane
    • 2x filter paper
    • Scotch-Brite Pad
    • Anode (+++)

    Note: Blotter should be firmly packed. If two membranes are to be blotted, repeat the above transfer sandwich. If only one gel is to be blotted, fill the space with more filter paper and another Scotch-Brite Pad.

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 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 as follows:
   - Anode (+++)
   - Filter paper
   - Membrane
   - Gel
   - Filter paper
   - Cathode (– – –)
4. Transfer the blot for 30 minutes at 20V.
5. Remove the gel from the sandwich and rinse with transfer buffer.
6. Use a cotton swab to remove any adhering gel from the membrane.

Staining Membranes

The MemCode™ Reversible Protein Stain Kits (Product No. 24580 for nitrocellulose; Product No. 24585 for PVDF) contain Reversible Protein Stain, Destain and Stain Eraser. Sensitivity is ~25 ng 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-HEPES-SDS Running Buffer (10X)	
Tris Base (MW = 121)	121 g
HEPES (free acid MW = 238)	238 g
SDS (MW = 288)	10 g
Add ultrapure water to 1 L	

- Before use dilute 10-fold with water. The pH of the 1X buffer should be ~8.0; do not adjust pH.
- Final composition of the 1X buffer is 100 mM Tris, 100 mM HEPES, 1% (~3 mM) SDS, pH 8.0.
- Do not use Tris-glycine-SDS running buffer with Precise Protein Gels.

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

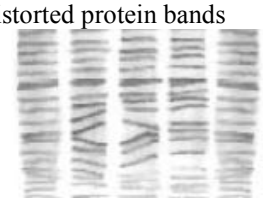
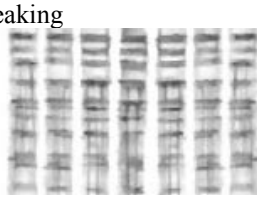
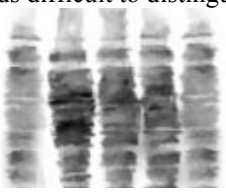
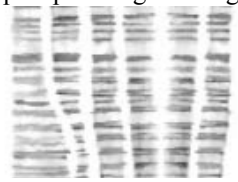
*Add if cleavage of disulfide bonds is desired.

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

Note: The pH of the buffer should be ~8.0

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

Troubleshooting

Problem	Cause	Solution
Distorted protein bands 	Air bubbles in the sample wells, or between gel and cassette, or at the bottom of the cassette	Use a transfer pipette to displace the air bubbles from the sample wells
	Sample contains appreciable carbohydrate	Remove the carbohydrate by enzymatic or chemical means
	Sample contains lipoproteins	Use a gel with a large pore size at top or try adding a non-ionic detergent
Streaking 	Poorly soluble or weakly charged particles (such as carbohydrates) in 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 2-D separation may be required
Sample spreading across gel 	Excess salt in the sample	Reduce salt by dialysis or ultra-filtration
	Too much protein 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 present in the gel	Wash gel extensively (3 × 5 minutes) with ultrapure water and use 30% methanol to destain gel
	Protein bands are diffusing	Use 10% TCA to fix the proteins in the gel
Buffer front and proteins migrate only partly down the gel and are distorted	Incorrect running buffer used (e.g., Tris-glycine-SDS)	Use only Tris-HEPES-SDS Running Buffer (100 mM Tris, 100 mM HEPES, 1% SDS, pH 8.0)

Related Products

26800 **PAGE Prep Protein Clean-Up and Enrichment Kit**

26691 **3-Color Prestained Molecular Weight Markers,** 1 × 48 tubes

26681 **Prestained Molecular Weight Markers,** 1 × 48 tubes

26651 **Chemiluminescent Molecular Weight Markers,** 1 × 48 tubes

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

77720 **BondBreaker® TCEP (Odorless reducing agent),** 5 ml

28398	**BupH Tris-HEPES-SDS Running Buffer,** 10 packs
28380	**BupH Tris-Glycine Transfer Buffer,** 40 packs
24590	**GelCode Blue Stain Reagent,** 500 ml
24612	**Silver Stain Kit II**
24597	**Color Silver Stain Kit**
24582	**E-Zinc® Reversible Stain Kit**

Please see our web site or catalog for more information on our complete line of Western blotting products, such as the following:

- 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

Bio-Rad®, Mini-PROTEAN® and Mini-Trans-Blot® are trademarks of Bio-Rad Laboratories.
Novex®, Surelock & Xcell II™ are trademarks of Novel Experimental Technology.
Mighty-Small™ is a trademark of Pharmacia-Hoefer Inc.
Scotch-Brite™ is a trademark of 3M Corporation.

This product ("Product") is warranted to operate or perform substantially in conformance with published Product specifications in effect at the time of sale, as set forth in the Product documentation, specifications and/or accompanying package inserts ("Documentation") and to be free from defects in material and workmanship. Unless otherwise expressly authorized in writing, Products are supplied for research use only. No claim of suitability for use in applications regulated by FDA is made. The warranty provided herein is valid only when used by properly trained individuals. Unless otherwise stated in the Documentation, this warranty is limited to one year from date of shipment when the Product is subjected to normal, proper and intended usage. This warranty does not extend to anyone other than the original purchaser of the Product ("Buyer").

No other warranties, express or implied, are granted, including without limitation, implied warranties of merchantability, fitness for any particular purpose, or non infringement. Buyer's exclusive remedy for non-conforming Products during the warranty period is limited to replacement of or refund for the non-conforming Product(s).

There is no obligation to replace Products as the result of (i) accident, disaster or event of force majeure, (ii) misuse, fault or negligence of or by Buyer, (iii) use of the Products in a manner for which they were not designed, or (iv) improper storage and handling of the Products.

Current versions of product instructions are available at *www.thermo.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.

Pierce Biotechnology 3747 N. Meridian Road | PO Box 117 Rockford, IL 61105 USA | (815) 968-0747 (815) 968-7316 fax | www.thermo.com/pierce