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ProSieve™ 50 Gel Solution

Introduction

ProSieve™ 50 Gel Solution is a unique, modified acrylamide formulation developed for high performance protein gel electrophoresis. Gels prepared from this solution have several advantages over conventional polyacrylamide gels. Specifically, when cast at a single concentration and used with a Tris/Tricine electrode buffer, ProSieve™ 50 Gels will separate proteins as gradient polyacrylamide gels (see Table below). Alternately, 8% ProSieve™ 50 Gels, when used with a Tris/Glycine electrode buffer, will easily separate proteins larger than 200 kDa.

ProSieve™ 50 Gels have a uniform pore size distribution allowing for faster protein mobility than in conventional polyacrylamide gels. These gels are also stronger, less likely to tear, and easier to handle.

Table 1: Gel Percentage Guidelines for Gradient Gel Separation Performance in Tris/Tricine Electrode Buffer

Protein Size Separation Range	**8%**	**10%**	**12%**	**14%**
Optimal Size Range (kDa)	25-150	15-100	10-70	5-50
Resolvable Size Range (kDa)	15-300	5-200	5-150	2-100

Materials

50% ProSieve™ 50 Gel Solution

Storage

ProSieve™ 50 Gel Solution should be stored at room temperature. It is stable for 24 months from the date of manufacture.

Reagents and solutions not provided

- 1.5 M Tris-HCI, pH 8.8
- 1 M Tris-HCI, pH 6.8
- 10% (w/v) SDS solution
- 0.1% (w/v) Bromophenol Blue
- Ammonium Persulfate
- TEMED (N,N,N´,N´-tetramethylenediamine)
- Deionized Water
- Tris/Tricine Electrode Buffer or
- Tris/Glycine Electrode Buffer

Precautions

Wear gloves and use all safety precautions routinely used when handling acrylamide solutions. Material Safety Data Sheets (MSDS) for this product are available by contacting our Customer Service Department at 1-800-638-8174 or on our website at www.lonza.com.

Reagent Preparation

Electrode Buffers

10X Tris/Tricine/SDS Electrode Buffer

(Dilute to 1X with deionized water prior to use):

Tris Base	121.0 g
Tricine	179.0 g
10% SDS solution*	100 ml
Deionized Water to	1000 ml

Do not adjust the pH.
Store at room temperature.
*Omit for native proteins.

10X Tris/Glycine/SDS Electrode Buffer

(Dilute to 1X with deionized water prior to use)

Tris Base	30.0 g
Glycine	144.0 g
10% SDS solution*	100 ml
Deionized Water to	1000 ml

Do not adjust the pH.
Store at room temperature.
*Omit for native proteins.

2X Denaturing Sample Buffer

0.5 M Tris-HCI, pH 6.8	2.5 ml
Glycerol	2.0 ml
10% SDS solution*	4.0 ml
0.1% (w/v) Bromophenol Blue	0.5 ml
2-mercaptoethanol*	0.5 ml
Deionized Water to	10 ml

Store at -20°C.
*Omit for native proteins.
Caution: 2X Sample Buffer containing 2-mercaptoethanol should be prepared in a fume hood.
0.2 M (final concentration) Dithiothreitol (DTT) may be used in place of 2-mercaptoethanol.

Gel preparation and pouring

Table 1: Preparation of a 10 ml Resolving Gel
(Proportionally adjust volumes for larger gels.)

Components	*8% Gel*	*10% Gel*	*12% Gel*	*14% Gel*
Deionized Water	5.7 ml	5.3 ml	4.9 ml	4.5 ml
ProSieve™ 50 Gel Solution	1.6 ml	2.0 ml	2.4 ml	2.8 ml
1.5M Tris-HCl, pH 8.8	2.5 ml	2.5 ml	2.5 ml	2.5 ml
10% SDS Solution‡	0.1 ml	0.1 ml	0.1 ml	0.1 ml
10% APS*	100 µl	100 µl	100 µl	100 µl
TEMED	4.0 µl	4.0 µl	4.0 µl	4.0 µl

*APS solutions should be prepared fresh just prior to use.

‡Omit for native protein analysis.

1. Clean your glass plates and wipe them with ethanol. Allow them to air-dry.
2. Assemble the gel cassette according to manufacturer's instructions or use preassembled cassettes.
3. Prepare the resolving gel. Place the specified quantities of the first five components from **Table 1** in a clean beaker. Thoroughly mix the components.

NOTE: The ProSieve™ 50 Gel Solution does not require degassing. If required by your procedure, degas the solution prior to the addition of SDS.

4. Add the specified amount of TEMED and mix the solution by inversion or gentle swirling.
5. Pour the gel solution between the cassette glass plates. Carefully layer water or water-saturated n-butanol along the surface of the gel solution with a pipette. Allow the gel to polymerize for at least 30 minutes at room temperature before casting the stacking gel.
   NOTE: Resolving gels can be stored for up to one week at 4°C. To prepare the gel for storage, decant the overlay and thoroughly rinse the top of the gel with 1X electrode buffer. Keep the gels moist by layering a small volume of 1X electrode buffer on top of the gel and wrapping the gel in plastic wrap.
6. Decant the water or n-butanol prior to casting the stacking gel. Thoroughly rinse the top of the gel with 1X electrode buffer and decant. (If n-butanol was used as an overlay, several washings will be required.) Make sure that all of the overlay is removed completely by aspirating or by absorbing excess fluid with a paper towel or blotting paper.
7. Prepare the stacking gel solution by combining the specified quantities from **Table 2** into a clean beaker. Thoroughly mix the components.

Table 2: Preparation of a 5% Stacking Gel

Components	**1 ml**	**5 ml**
Deionized Water	750 µl	3.75 ml
ProSieve™ 50 Gel Solution	100 µl	0.5 ml
1M Tris-HCl, pH 6.8	130 µl	0.65 ml
10% SDS Solution‡	10 µl	50 µl
10% APS*	10 µl	50 µl
TEMED	1 µl	5.0 µl

*APS solutions should be prepared fresh just prior to use.
‡Omit for native protein analysis.

8. Pour the stacking gel solution on top of the resolving gel with a pipette. Insert the comb and add sufficient stacking gel solution to completely fill the cassette. Allow the stacking gel to polymerize for 30 to 40 minutes at room temperature.
   NOTE: Stacking gels should be prepared fresh prior to use. If necessary, polymerized gels can be stored with the comb in place and the gel wrapped with plastic wrap at 4°C for up to 16 hours with no loss in performance.

Sample Preparation

Combine equal volumes of your protein sample with the 2X sample buffer in a microcentrifuge tube and place the tube into a boiling water bath for 2 to 3 minutes. After heating, briefly centrifuge the tube to collect droplets. For native proteins, do not apply heat.

1. Load your samples immediately or place them on ice until ready for use. Treated samples can be stored at -20°C.
   NOTE: The amount of sample to load on the gel depends on sample composition (e.g., complex protein mixtures or polypeptides) and the detection method. When Coomassie® Blue staining, load 1 µg-10 µg of a highly purified protein per lane or load 25 µg-50 µg of a complex mixture (cell lysates) per lane. When silver staining, load 10- to 100- fold less protein per lane.

Electrophoresis

1. Prepare enough working solution of 1X electrode buffer to completely fill the upper and lower buffer chambers of your electrophoresis apparatus.
2. Remove the comb from the stacking gel and thoroughly flush the wells with 1X electrode buffer.
3. Mount the gel cassette on the electrophoresis apparatus according to the manufacturer's instructions.
4. Fill the upper and lower buffer chambers with 1X electrode buffer. Flush the sample wells several times and fill with electrode buffer.
5. Load equal volumes of the samples on the gel (if possible). Load empty wells with an equal volume of 1X sample buffer. If lanes are left empty or loaded with unequal volumes, adjoining samples may spread during electrophoresis.
6. Electrophorese samples at 130 V (constant voltage) for minigels (10 cm x 10 cm x 0.1 cm) or at approximately 200 volts for larger gels (18 cm x 16 cm x 0.1 cm).
7. Stop the electrophoresis once the tracking dye reaches the bottom of the resolving gel. Approximate times for electrophoresis are:

   Minigels 60 minutes
   Large Gels 90 minutes
   (Tris/Tricine electrode buffer)

8. Remove the protein gel cassette from the apparatus and discard the electrode buffer. Carefully separate the two plates while maintaining gel orientation. Trim one corner of the gel so sample order is not lost during drying or staining.
9. Remove the gel from the plate and proceed with protein detection or transfer.

Protein Detection

Conventional protein detection techniques used for polyacrylamide gels are compatible with gels prepared from the ProSieve™ 50 Gel Solution.

Approximate Detection Limits

Stain	Coomassie® Brilliant Blue	Silver Stain
Sensitivity	0.3 µg-1.0 µg per protein band	2.0 µg -5.0 µg per protein band

Blotting and Drying

Conventional techniques for blotting, drying, and preserving polyacrylamide gels are compatible with ProSieve™ 50 Gels.

Ordering Information

Catalog No.	Description	Size
50617	ProSieve™ 50 Gel Solution	125 ml
50618	ProSieve™ 50 Gel Solution	250 ml

Related Products

PAGEr™ Precast Gels
ProSieve™ Color Protein Markers
ProSieve™ Protein Markers
SYPRO® Red Gel Stain
SYPRO® Orange Gel Stain
SYPRO® Tangerine Gel Stain
AccuGENE™ Tris-Glycine Buffer
AccuGENE™ Tris-Glycine SDS Buffer

ProSieve™ 50 Gel Solution is intended For Research Use Only.

For more information contact Scientific Support at (800) 521-0390 or visit our website at www.lonza.com.