SurePAGE™, Bis-Tris, 10 cm × 8cm gels

Version: 08/28/2017

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I. INTRODUCTION

GenScript SurePAGE, Bis-Tris, 10 cm x 8 cm gels are high-performance precast mini polyacrylamide gels with a special design that allows large sample loading volumes. The unique formulation of the gel and cassette design enables superior band resolution and significantly improved band evenness. SurePAGE gels are cast in a neutral pH buffer that minimizes polyacrylamide hydrolysis, increases gel stability and minimizes protein modification.

SurePAGE gels guarantee excellent batch-to-batch consistency and a reliable protein migration pattern. With specially formulated Tris-MOPS or Tris-MES running buffer, proteins can be separated quickly and efficiently for subsequent detection by staining or Western blotting.

SurePAGE, Bis-Tris, 10x8 gels are available in gradient (4-20%, 4-12%, and 8-16%) and homogeneous (8%, 10%, and 12%) concentrations. Each gel concentration has comb configurations of 10-well, 12-well and 15-well.

Key Features and Benefits:

- **Large loading volume**–Up to 80 µl per well
- **Easy to use** – Wider well opening allows sample loading with regular pipette tips
- **High resolution** – More even, sharp bands
- **Long shelf life** – Up to 12 months if stored at 2-8℃
- **Compatible cassette design** – Fits all popular mini-gel tanks
- **High reproducibility** – Guaranteed consistent performance of each gel
- **Cost effective** – Significant price reduction compared to other competitors
- **Outlined and numbered wells**: Each well is outlined and numbered for easier sample identification

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II. GEL SELECTION GUIDE

Table 1. Gel Selection Guide

Cat.No.	%Acrylamide	No. of Wells	Max. Well Vol.	Running Buffer	Transfer Buffer
M00652	4-12%	10	80μl	MOPS/MES	Tris-Bicine
M00653	4-12%	12	60μl	MOPS/MES	Tris-Bicine
M00654	4-12%	15	40μl	MOPS/MES	Tris-Bicine
M00655	4-20%	10	80μl	MOPS/MES	Tris-Bicine
M00656	4-20%	12	60μl	MOPS/MES	Tris-Bicine
M00657	4-20%	15	40μl	MOPS/MES	Tris-Bicine
M00658	8-16%	10	80μl	MOPS/MES	Tris-Bicine
M00659	8-16%	12	60μl	MOPS/MES	Tris-Bicine
M00660	8-16%	15	40μl	MOPS/MES	Tris-Bicine
M00661	8%	10	80μl	MOPS/MES	Tris-Bicine
M00662	8%	12	60μl	MOPS/MES	Tris-Bicine
M00663	8%	15	40μl	MOPS/MES	Tris-Bicine
M00664	10%	10	80μl	MOPS/MES	Tris-Bicine
M00665	10%	12	60μl	MOPS/MES	Tris-Bicine
M00666	10%	15	40μl	MOPS/MES	Tris-Bicine
M00667	12%	10	80μl	MOPS/MES	Tris-Bicine
M00668	12%	12	60μl	MOPS/MES	Tris-Bicine
M00669	12%	15	40μl	MOPS/MES	Tris-Bicine

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The protein migration table below can help you to choose the appropriate gel for your protein electrophoresis. For best results select a gel that allows the best separation for your sample's molecular weight range. For a wide range of molecular weights you may want to select a gradient gel.

Table 2. Protein Migration Table with MOPS running buffer

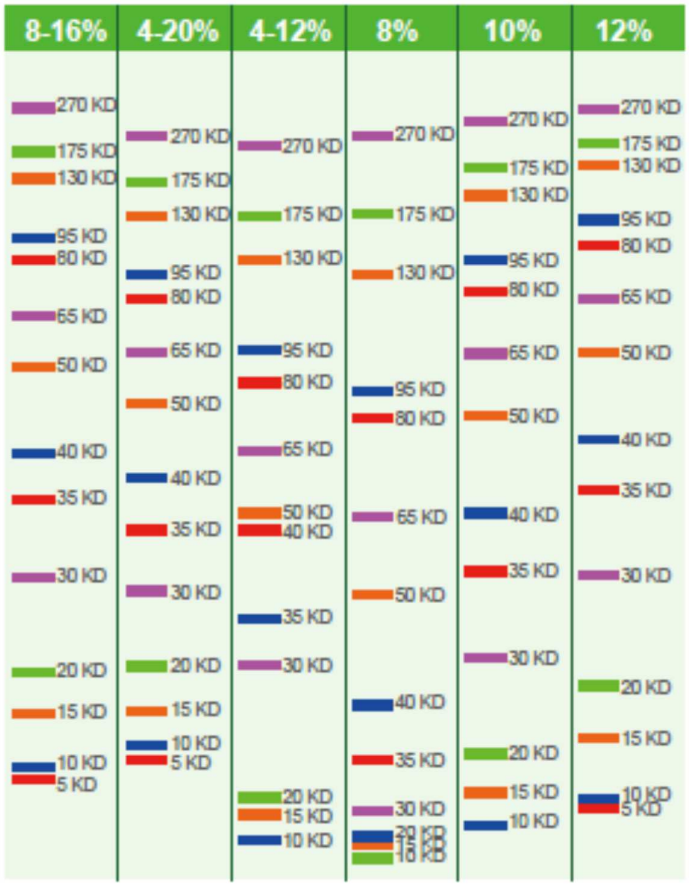

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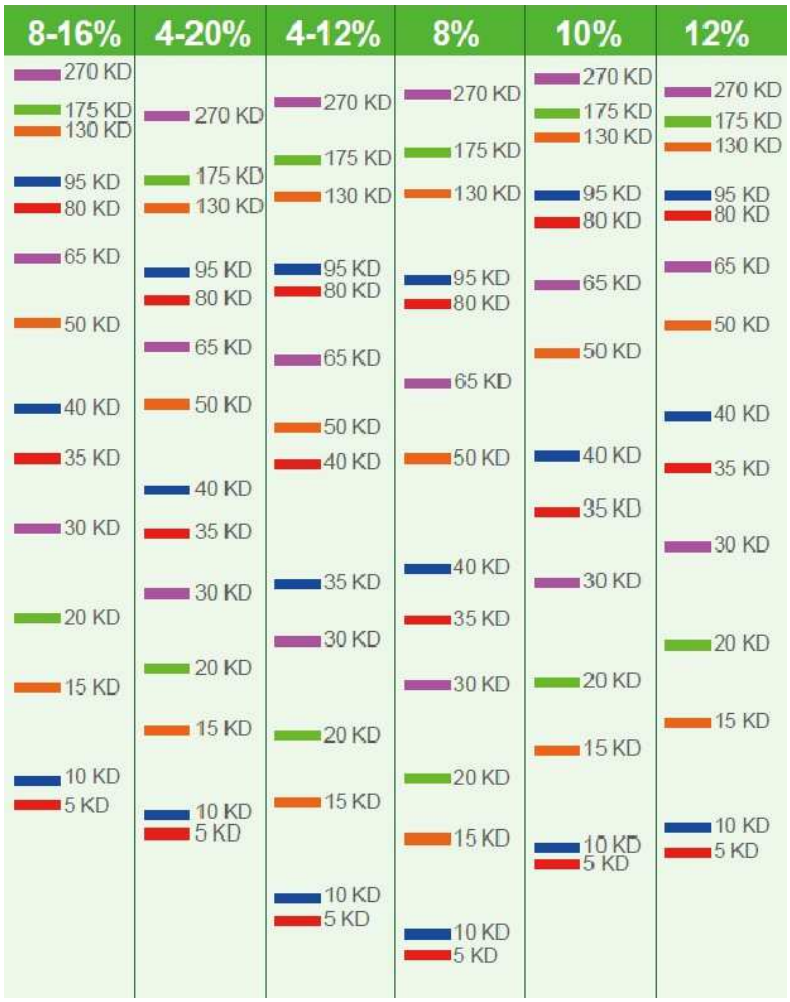
Table 3: Protein Migration Table with MES running buffer (M00677). Use MES buffer for separation of smaller proteins (<50kD).



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III. COMPATIBLE GEL TANKS

SurePAGE, Bis-Tris, 10x8 are compatible with the following Gel Tanks:

*Bio-Rad Mini-PROTEAN® II & 3**

*Bio-Rad Mini-PROTEAN®Tetra System**

*Invitrogen Novex XCell I, II, & Surelock® (**Use with GenScript Gel Tank Adapter Plates**)*

LONZA PAGEr® Minigel Chamber

Hoefer Mighty Small (SE 260/SE 250)

Hoefer Tall Mighty Small (SE 280)

**please reverse the gasket, see instructions below*

IV. Instructions for using SurePAGE, Bis-Tris, 10x8 gels

A. Prepare the Gel Buffer and Gel Tank

1. Dissolve one pack of Tris-MOPS-SDS Running Buffer Powder (Cat. No. M00138) in 1 L deionized water to make 1 L 1x MOPS running buffer.
   If you're using MES buffer, dissolve one pack of MES SDS Running Buffer Powder (Cat. No. M00677) in 1 L deionized water to make 1 L 1x MES running buffer.
   Refer to Section B for recipes of MOPS or MES running buffer.
2. Remove SurePAGE, Bis-Tris, 10x8 gel from the package, peel off the sealing tape at the bottom of the gel cassette (see Figure 1).

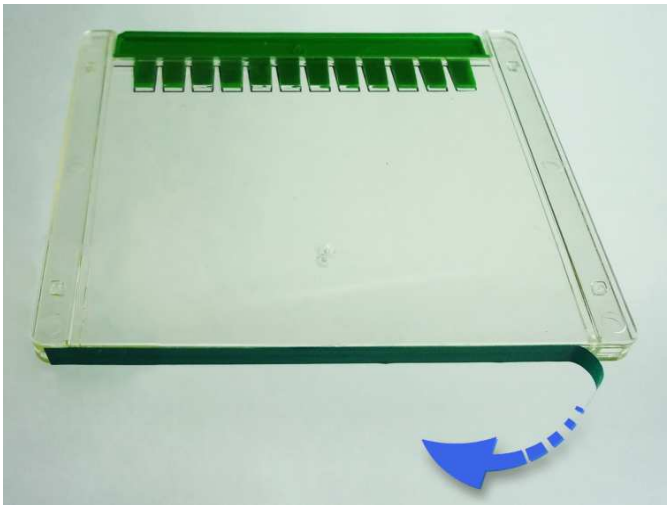

Figure 1. Peel the tape off from the bottom of the cassette

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3. Gently remove the comb from the gel cassette (see Figure 2).

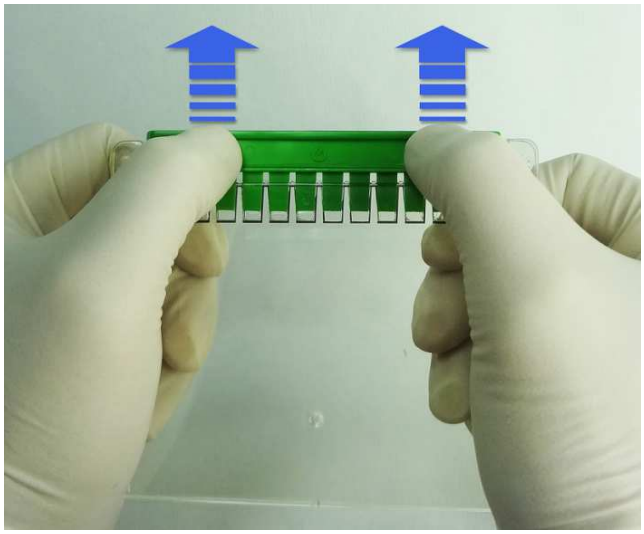

Figure 2. Remove the comb from the gel cassette

4. Insert the gel into the gel running apparatus.
   Refer to the apparatus manufacturer's instructions.

Notes for Using Bio-Rad Mini-PROTEAN® Tetra System: remove the gasket from the inner electrode assembly, flip it around so the flat side of the gasket is facing outwards and insert the gasket back into the inner electrode assembly (see Figure 3).

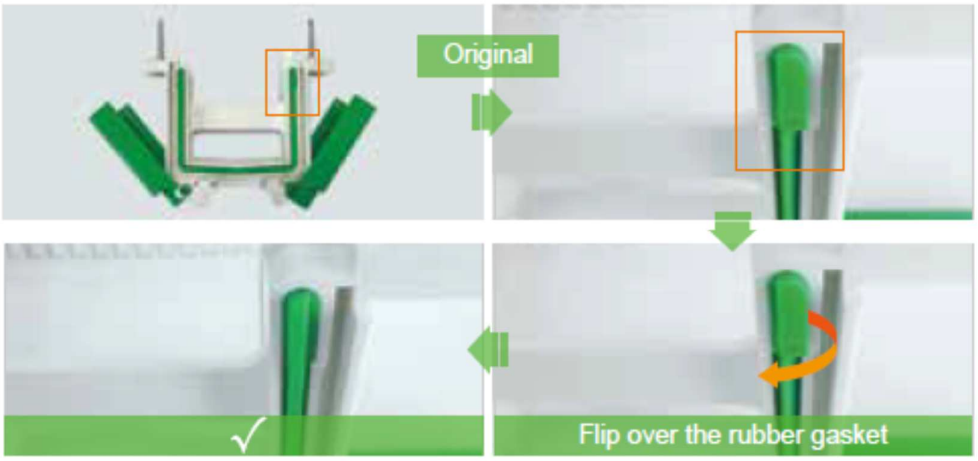

Figure 3. Use of SurePAGE, Bis-Tris, 10x8 in Bio-Rad Mini-PROTEAN® Tetra System

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Notes for using Invitrogen Novex Mini-Cell tanks: Adapters provided in the package are needed since the SurePAGE, Bis-Tris, 10x8 cassette is thinner than the Invitrogen NuPAGE® gel cassette. Each gel needs one adaptor.

See figure 4 for use of SurePAGE, Bis-Tris, 10x8 in the Invitrogen Novex® Mini-Cell.

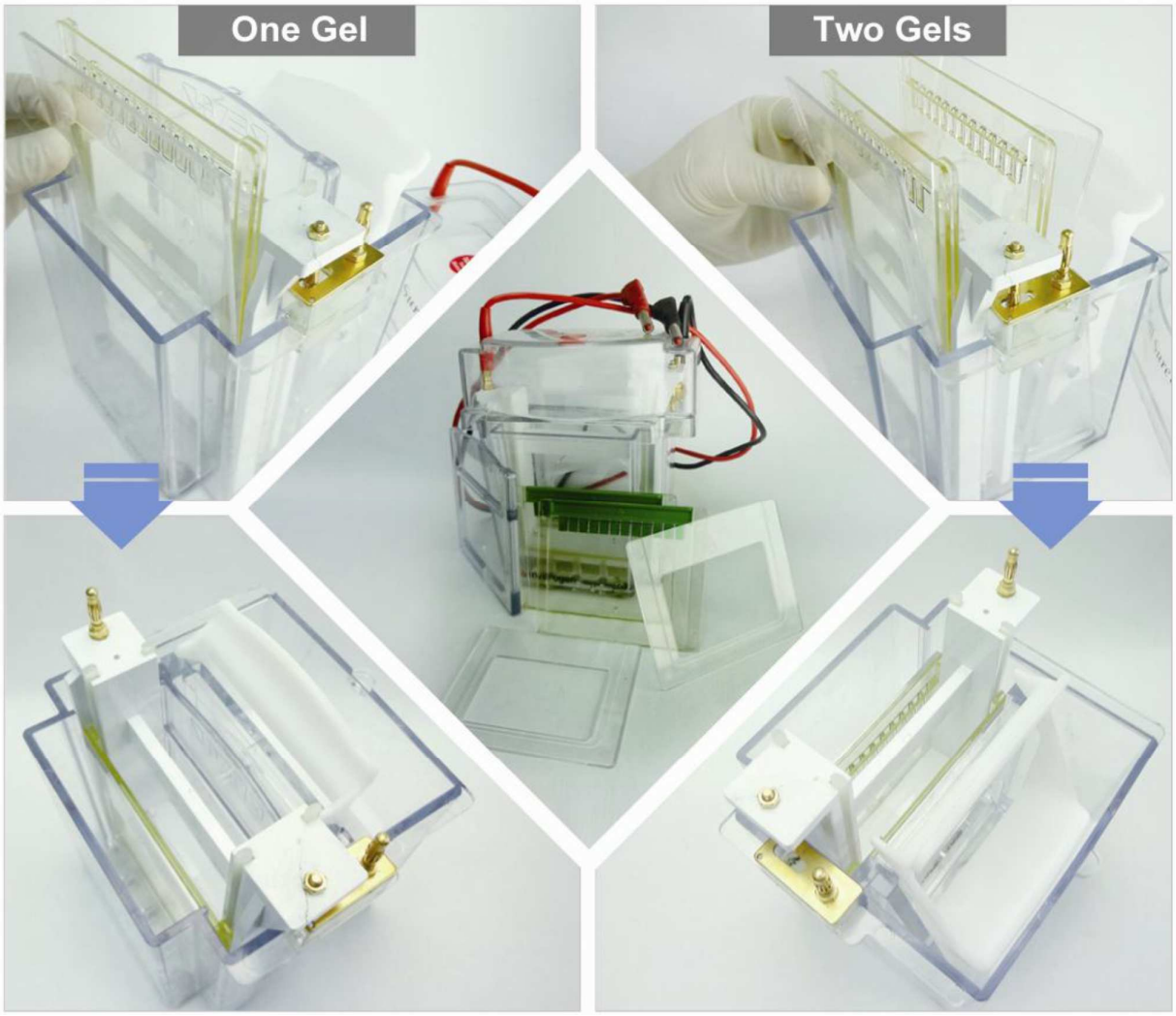

Figure 4. Use of SurePAGE, Bis-Tris, 10x8 in Invitrogen Novex® Mini-Cell

5. Pour sufficient 1x MOPS or MES running buffer into the inner tank of the gel running apparatus until the buffer is above the top of the comb. Fill the outer tank with the same running buffer to ensure proper cooling. For best results, the buffer level in the outer tank should be above the top of the sample wells.
   (**NOTE:** Do **NOT** use Tris-glycine running buffer for SurePAGE, Bis-Tris, 10x8.)

6. Rinse the sample wells thoroughly with 1x running buffer to remove air bubbles and replace any storage buffer.

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B. Prepare the sample

1. Sample preparation

For best result, we recommend using 4X LDS sample buffer (M00676) as the sample loading buffer. An alternative SDS sample buffer is 5x sample buffer (MB01015). Please refer to the table below on buffer and sample preparation.

With 4X LDS sample buffer (**recommended**) (M00676)

Reagent	Volume
Sample	x
4X LDS sample buffer	2.5µl
1M DTT (10X)	1µl
Deionized Water	To 6.5µl
Total Volume	10µl

Heat samples at **70°C** for 10 minutes before loading.

Buffer formulations

4X LDS sample buffer:

Tris HCl	0.666g
Tris Base	0.682g
Lithium dodecyl sulfate (LDS)	0.800g
EDTA	0.006g
Glycerol	4g
SERVA Blue G250 (1% solution)	0.75ml
Phenol Red (1% solution)	0.25ml
Deionized water	to 10 ml

Store at +4˚C. The buffer is stable for 6 months when stored at +4˚C.

The pH of the 1X solution is 8.5. Do not adjust the pH with acid or base.

1× MES running buffer:

Tris base	6.06 g
MES	9.76g
SDS	1.0g
EDTA	0.3g
Deionized water	to 1000 ml

10x MOPS running buffer:

Tris base	60.6 g
MOPS	104.6g
SDS	10.0g
EDTA	3.0g
Deionized water	to 1000 ml

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2. Running the sample

   Protein sample loading.

   Make sure the loading tip is inserted vertically into the loading well for optimal results.

Figure 5. Sample loading

Note: Optimal sample volume should be established by trial and error. Protein overloading will cause smearing and band distortion. Overloading with samples containing high content of free carbohydrates may also lead to band distortion or prevent the protein from entering the gel (See Troubleshooting).

Place the electrical cover onto the gel running cassette and plug the electrical leads into the power supply (red to red and black to black). Run the gel at 140 volts for 45-55 minutes until the dye front reaches the bottom of the gel, depending on the sizes of the proteins of interest (Table 3).

Table 3. Electrophoresis conditions for SurePAGE, Bis-Tris, 10x8, **one gel**

Voltage	Start	Finish	Run Time per Gel*
140 V (Recommended)	75-100 mA	30-50 mA	45-55 minutes
* The run time here is under laboratory temperature of 20°C with Tris-MOPS-SDS buffer. Gel running time varies under different laboratory temperatures.			

Important notes:

- Make sure to use a compatible gel tank. Leaking between the inner and outer tank will cause slow migration rate. (See Troubleshooting)
- The running time may also vary depending on your power supply and gel concentration.

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3. Removing the gel from the Cassette (see Figure 6)
    a. Once electrophoresis is finished, remove the gel cassette from the gel tank.
    b. There are three contact points between the two plates on each side of the gel cassette. Open the gel cassette by carefully inserting the cassette opener into the gap between the two plates and flanking one of the contact points.
    c. Gently wiggle the cassette opener **up and down** to separate the two plates. Repeat the operation along both sides of the cassette until the two plates are completely separated. A cracking sound may be heard as you open the cassette. It is possible for the gel cassette to crack while opening it. Please wear goggles for protection.
    d. Upon opening, carefully remove and discard the plates and place the gel in water. Please dispose the used cassettes as non-hazardous medical waste.

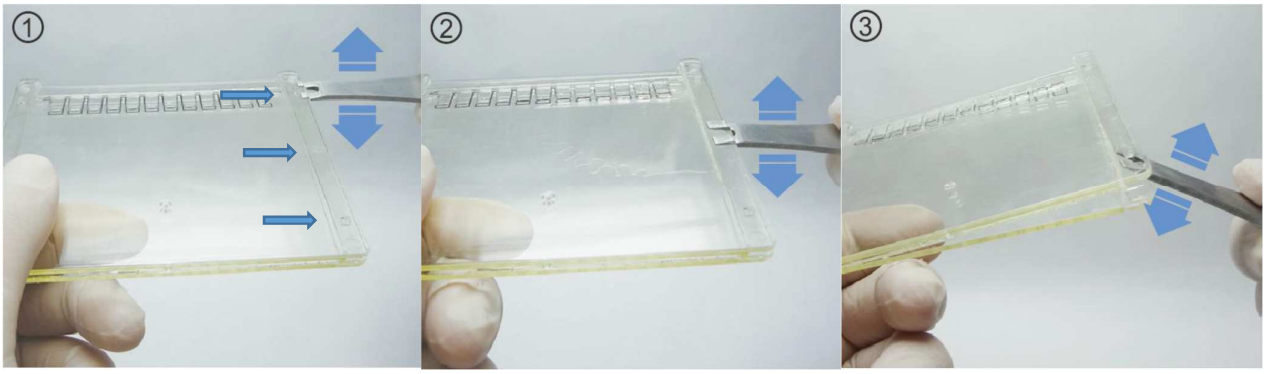

Figure 6. Open the gel cassette and remove the gel.

C. Storage

Gels are stable for up to 12 months if stored at 2-8°C. See package cover for expiration date.

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V. STAINING

All standard SDS PAGE gel staining procedures can be used with SurePAGE, Bis-Tris, 10x8. When using commercially available staining reagents and devices, follow the manufacturer's instructions.

eStain® L1 Staining (Cat No. L00657)

ExpressPlus and SurePAGE gels can be stained using GenScript's eStain® L1 Protein Staining System which allows quick staining of gels in 10 minutes. Check the website for more details: http://www.genscript.com/eStain-L1-protein-staining-system.html

VI. PROTEIN TRANSFER

All standard transferring procedures can be used with SurePAGE, Bis-Tris, 10x8. We recommend Tris Bicine transfer buffer (Cat. No. M00139)

If you are transferring your gel, DO NOT STAIN the gel before the transfer.

VII. Examples

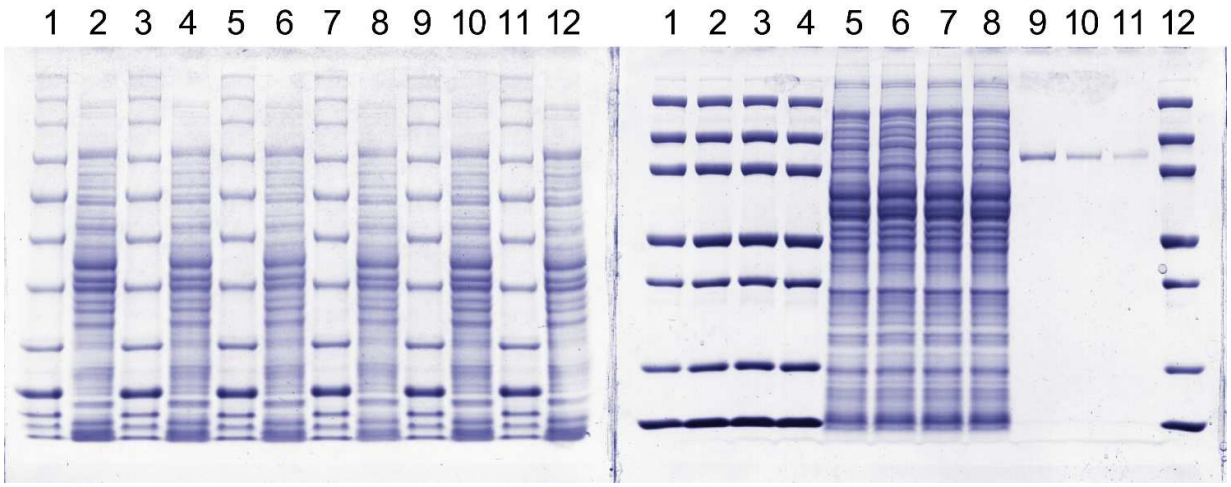

Figure 7. Protein separation using 4-12% SurePAGE, Bis-Tris, 10x8

Proteins were separated on a 12-well, 4-12% SurePAGE, Bis-Tris, 10x8 (L) and a 12-well, 4-12% SurePAGE, Bis-Tris, 10x8 (R) and then stained using the eStain® L1 Protein Staining System (R-250).
(L): Lane 1, 3, 5, 7, 9, 11: 4 µl Color Prestained Protein Standard, Broad Range (11-245kDa) (P7712S);
Lane 2, 4, 6, 8, 10, 12: 6 µl *E.coli* cell lysate.
(R): Lane 1, 2, 3, 4, 12: 4 µl GenScript PAGE-MASTER Protein Standard (for SDS-PAGE) (M00516);
Lane 5, 6, 7， 8: 6 µl *E.coli* cell lysate;
Lane 9, 10, 11: 50 ng/ 25 ng/ 12.5 ng BSA.

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VIII. TROUBLESHOOTING

Problems	Probable cause	Solution
Distorted protein bands	Air bubbles in the sample wells	Use a syringe or a pipette to flush the sample wells thoroughly with running buffer before sample loading
Some part of the tracking dye changed to yellow	Buffer enters gel because of broken cassette	Gel tank is not compatible or cassette was damaged
	pH value decreased	Prepare new running buffer with deionized water. Check pH
Streaking	Insoluble or weakly charged particles (such as carbohydrates) in sample	Heat sample in the presence of SDS, centrifuge sample and load the supernatant
Electrophoresis time is too long	Seal is not removed from the bottom of the cassette	Peel the seal off from the bottom of cassette before loading
	Incorrect running conditions	Use fixed voltage and automated current, e.g. 140V throughout the electrophoresis
Bands are not well separated	Incorrect gel percentage	Use the protein migration table to choose the appropriate gel
	Sample overloading	Reduce sample loading amount, especially when the sample contains many kinds of protein.
	Insufficient SDS in loading buffer	Increase SDS content in the sample during preparation
	Insufficient buffer to keep tank cool	Add more buffers to the outer tank until it's at the same level or above the top of the sample wells
Sample spreading across the gel	Sample contains too much salt	Reduce salt content by dialysis or ultra-filtration
The voltage cannot reach the set value	Leaking between the inner and outer tank during run	Use compatible gel tank
	Excess salt in the sample	Reduce salt content by dialysis or ultra-filtration
Lots of air bubbles between the gel and the cassette	Running buffer is hot after electrophoresis	Add more running buffer to the outer tank

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IX. RELATED PRODUCTS AND ORDER INFORMATION

Product	Cat. No.
4x LDS sample buffer	M00676
5x Sample Buffer	MB01015
MES SDS Running Buffer Powder	M00677
Tris-MOPS-SDS Running Buffer Powder	M00138
Tank Adaptor (for use with Novex gel tanks)	L00671
Cassette opener	L00674
Buffer Dam	L00699
Transfer Buffer Powder	M00139
eStain® L1 Protein Staining Device	L00657
eStain® L1 Protein staining kit	L00659-1
Broad Multi Color Pre-Stained Protein Standard	M00624
Smart Advanced Broad-Range Protein Standard	M00441
Smart Dual Color Pre-Stained Protein Standard	M00442
Smart Multi Color Pre-Stained Protein Standard	M00443
Protein Marker for Fluorescent Western Blotting	M00124
PAGE-MASTER Protein Standard (for SDS-PAGE)	M00516
PAGE-MASTER Protein Standard Plus	MM1397-500
WB-MASTER Protein Standard	M00521

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