ExpressPlus™ PAGE Gels, 10×10

Version: 03072016

I. INTRODUCTION

GenScript ExpressPlus™ PAGE Gels are high-performance precast mini polyacrylamide gels specially designed for large loading volumes. The unique design of the cassette gives better band resolution and significantly improves the sample distribution in the loading wells which increases the evenness of the band. ExpressPlus™ PAGE Gels are casted in a neutral pH buffer that minimizes the hydrolysis of polyacrylamide and results in extra gel stability.

Manufactured without SDS, ExpressPlus™ PAGE Gels are ideal for SDS-PAGE and native electrophoresis depending on the running buffer and transfer buffer used. The proprietary gel-casting techniques provide excellent batch-to-batch consistency and guarantee a reliable migration pattern. Using specially formulated Tris-MOPS running buffer, ExpressPlus™ PAGE Gels enable proteins to be separated quickly and easily for subsequent detection by staining or Western blotting.

The ExpressPlus™ PAGE Gels are available in gradient (4-20%, 4-12%, and 8-16%) and homogeneous (8%, 10%, and 12%) concentrations and in 10-well, 12-well and 15-well formats.

Key Features:

- **Large loading volume**–Up to 70 µl per well
- **Easy to use** – Wider 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℃
- **High reproducibility** – Guaranteed consistent performance of each gel
- **Cost effective**– Significant reduction in the cost of each experiment
- **Compatible with Novex system–**compatible with XCell SureLock™ and Bolt™ Mini Gel Tank

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GenScript ExpressPlus™ PAGE Gels Specifications

item	10×8 Gel	10×10 Gel
Gel material	Polyacrylamide	Polyacrylamide
Gel dimensions	7.4 cm×8.4 cm(H×W)	8.6 cm×8.0 cm(H×W)
Gel thickness	1.0 mm	1.0 mm
Resolving gel height	6 cm	6.5 cm
Cassette dimensions	8.4 cm×10.0 cm(H×W)	10.2 cm×10.0 cm(H×W)
Cassette material	Styrene copolymer	Acrylic
Comb material	Styrene copolymer	Polycarbonate
Storage conditions	Store flat between 2°C and 8°C; DO NOT FREEZE	Store flat between 2°C and 8°C; DO NOT FREEZE

Important note

Improper storage of ExpressPlus™ PAGE Gels can produce numerous artifacts. Gels should be stored flat between 2°C and 8°C. Avoid freezing or prolonged storage above 8°C. If you suspect your gels have been stored improperly, **THEY SHOULD BE DISCARDED.**

II. GEL SELECTION GUIDE

Table 1. Gel Selection Guide

Cat.No.	%Acrylamide	Wells	Running Buffer	Transfer Buffer	Separation Range
M00810L	8%	10	MOPS, MES	Tris-Bicine, Tris-glycine	250—15 kDa
M01010L	10%	10	MOPS, MES	Tris-Bicine, Tris-glycine	230—10 kDa
M01210L	12%	10	MOPS, MES	Tris-Bicine, Tris-glycine	200—6 kDa
M42010L	4-20%	10	MOPS, MES	Tris-Bicine, Tris-glycine	250—3.5 kDa
M81610L	8-16%	10	MOPS, MES	Tris-Bicine, Tris-glycine	230—6 kDa
M41210L	4-12%	10	MOPS, MES	Tris-Bicine, Tris-glycine	250—15 kDa
M00812L	8%	12	MOPS, MES	Tris-Bicine, Tris-glycine	250—15 kDa
M01012L	10%	12	MOPS, MES	Tris-Bicine, Tris-glycine	230—10 kDa
M01212L	12%	12	MOPS, MES	Tris-Bicine, Tris-glycine	200—6 kDa
M42012L	4-20%	12	MOPS, MES	Tris-Bicine, Tris-glycine	250—3.5 kDa
M81612L	8-16%	12	MOPS, MES	Tris-Bicine, Tris-glycine	230—6 kDa
M41212L	4-12%	12	MOPS, MES	Tris-Bicine, Tris-glycine	250—15 kDa
M00815L	8%	15	MOPS, MES	Tris-Bicine, Tris-glycine	250—15 kDa
M01015L	10%	15	MOPS, MES	Tris-Bicine, Tris-glycine	230—10 kDa
M01215L	12%	15	MOPS, MES	Tris-Bicine, Tris-glycine	200—6 kDa

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M42015L	4-20%	15	MOPS, MES	Tris-Bicine, Tris-glycine	250—3.5 kDa
M81615L	8-16%	15	MOPS, MES	Tris-Bicine, Tris-glycine	230—6 kDa
M41215L	4-12%	15	MOPS, MES	Tris-Bicine, Tris-glycine	250—15 kDa

Table 2. Recommended loading volume and amount

	Maximum sample volume per well	Recommended loading volume per well	Total amount of protein per well	Recommended amount of protein per well
10×10 Gel,10-well	70 µl	5-20 µl	60 µg	500 ng-1 µg
10×10 Gel, 12-well	45 µl	4-15 µl	50 µg	350 ng-1 µg
10×10 Gel,15-well	30 µl	3-10 µl	40 µg	200 ng-1 µg
10×8 Gel,10-well	80 µl	5-25 µl	60 µg	500 ng-1 µg
10×8 Gel,12-well	60 µl	4-20 µl	50 µg	350 ng-1 µg
10×8 Gel,15-well	40 µl	3-15 µl	40 µg	200 ng-1 µg

Total amount of protein was defined by BSA, the more kinds of protein in the sample, the less amount of protein you should run in a well. Too much protein cause low resolution. Please optimized by the method of trial and error.

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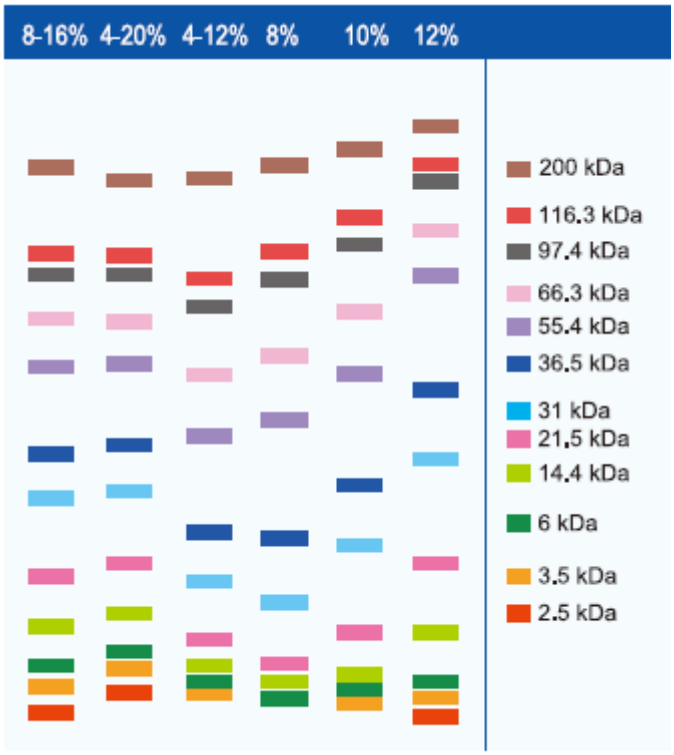
The protein migration table below can help you choose the appropriate gel for your protein electrophoresis analysis.

Table 3. Protein Migration Table



Values unit: Kilodaltons (kDa)

III. COMPATIBLE GEL TANKS

Unit	10×8 Gel	10×10 Gel
Bio-Rad Mini-PROTEAN® II & 3	Yes	Not comp.
Bio-Rad Mini-PROTEAN®Tetra System	Yes	Not comp.
LONZA PAGEr™ Minigel Chamber	Not comp.	Yes
Hoefer SE250 Mighty Small II Mini Vertical Electrophoresis Unit	Yes	Not comp.
Hoefer SE260 Mighty Small II Deluxe Mini Vertical Electrophoresis Unit)	Yes	Yes
Life Technologies Novex XCell Surelock®	Not comp.	Yes
Life Technologies Bolt™ Mini Gel Tank	Not comp.	Yes*

***Add a cushion into the bottom of Bolt™ Mini Gel Tank to create current circle.**

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IV. INSTRUCTIONS FOR USE

Precautions

Wear gloves and use all safety precautions when handling ExpressPlus™ PAGE Gels. Please read the Material Safety Data Sheet (MSDS) for this product prior to use. MSDS is available on our web site under www.genscript.com accompanying the ExpressPlus™ PAGE Gels.

A. Prepare Gel Buffer and Gel Tank

1. Dissolve one pack of MOPS Running Buffer Powder (Cat No. M00138) in 1 L deionized water to make 1 L 1x MOPS running buffer. Please refer to Section B for recipes of MOPS or MES running buffer.
2. Remove ExpressPlus™ PAGE Gel from the package, peel the sealing tape at the bottom of the gel cassette.
3. Remove the comb from the gel cassette gently.
4. Insert the gel into the gel running apparatus.
   Refer to the apparatus manufacturer's instructions.

Notes for using Life Technologies Bolt™ Mini Gel Tank: It is possible to use the Bolt™ Mini Gel Tank with ExpressPlus™ PAGE Gels. Please follow the instructions on the plastic insert provided in the package. (This cushion is **NOT** needed when using Novex XCell SureLock™ Gel Tank to run ExpressPlus™ PAGE Gels.)

See figure 1-3 for guidance of cushion in the Bolt™ Mini Gel Tank.

Figure 1. A cushion is needed to run a gel in Bolt™ Mini Gel Tank

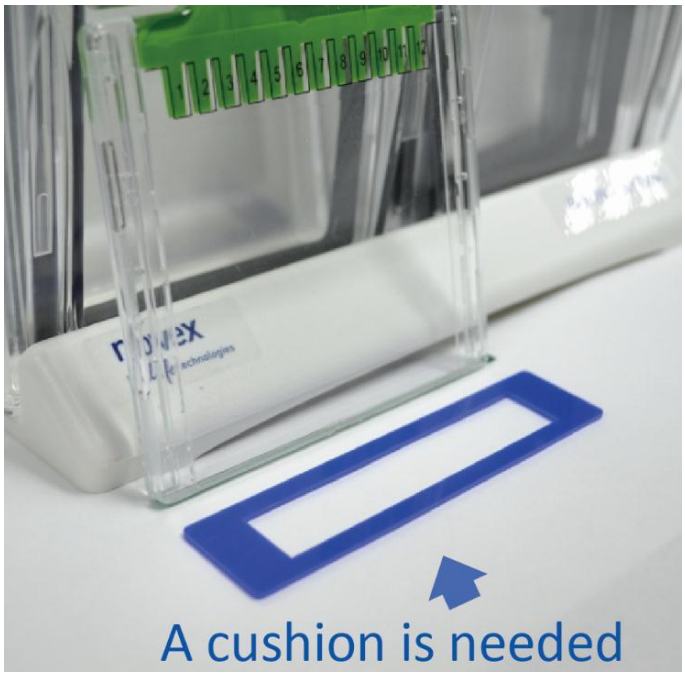

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Figure 2. Put the cushion into the bottom of Bolt™ Mini Gel Tank to create current circle

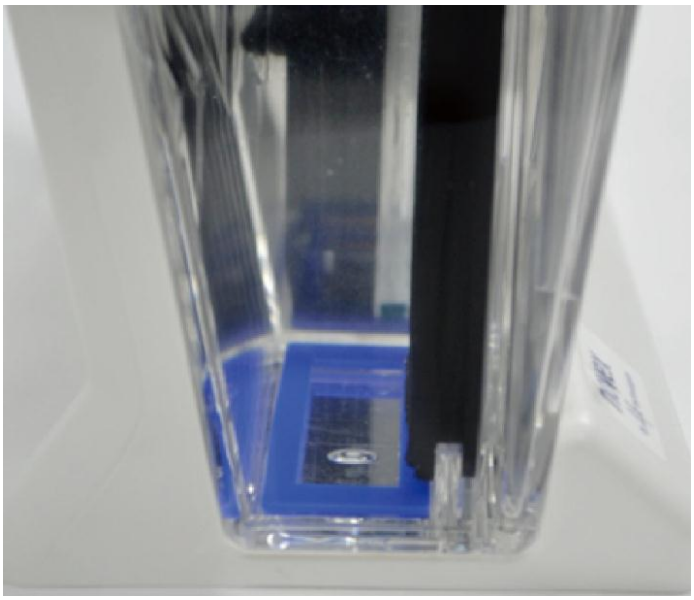
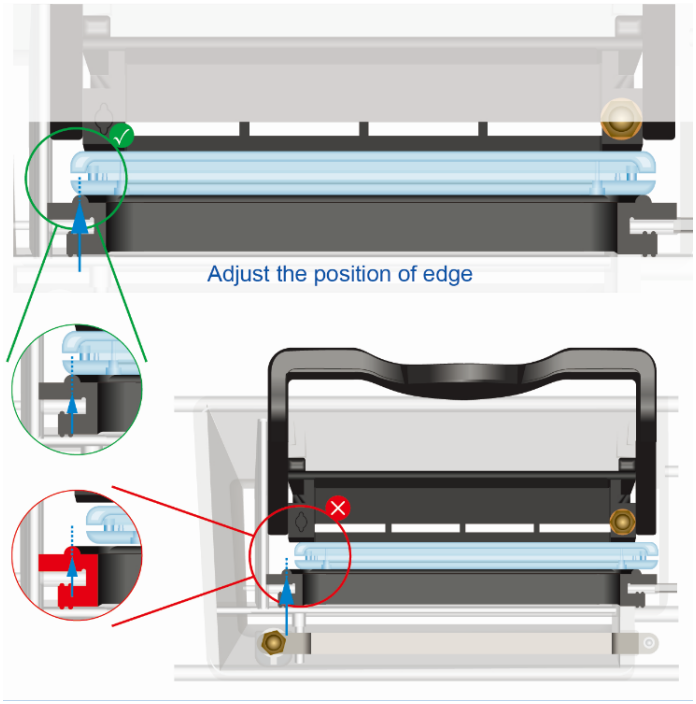


Figure 3. Adjust the edge of the cassette and the gasket to avoid buffer leaking



NOTE: 1. Check for leakage of inner chamber with running buffer or water.

2. In order to obtain more convenient operation, please upgrade your Bolt® Mini Gel Tank by replacing the current black cam handles (see figure 3) on your cassette clamps with new gray cam handles (Thermo Fisher Cat. No. A26732).

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5. Pour sufficient 1x MOPS or MES running buffer into the inner tank of the gel running apparatus to cover the sample wells by 5-7 mm. Fill the outer tank with the same running buffer to ensure proper cooling. For best results, the buffer in the outer tank should be above the top level of the sample wells. (**NOTE: DO NOT use tris-glycine running buffer** for ExpressPlus™ PAGE Gels.)
6. Rinse the sample wells thoroughly with 1x running buffer to remove air bubbles and displace any storage buffer.

B. Sample Running

1. For SDS PAGE

SDS Sample preparation

5x sample buffer:

SDS	1.0 g
Glycerol	5.0 ml
Bromophenol Blue	25 mg
Tris base	150 mg
2-Mercaptoethanol	1.0 ml
Deionized water (use 8 M NaOH or 8 M HCl adjust the pH to 6.8)	to 10 ml

10× MES running buffer:

Tris base	60.6 g
MES	97.6g
SDS	10g
EDTA	3g
Deionized water (use 8 M NaOH or 8 M HCl adjust the pH to 7.3)	to 1000 ml

10x MOPS running buffer:

Tris base	60.6 g
MOPS	104.6g
SDS	10g
EDTA	3g
Deionized water (use 8 M NaOH or 8 M HCl adjust the pH to 7.3)	to 1000 ml

1x protein sample buffer:

Sample	x µl
Sample buffer (5x)	2 µl
Deionized water	to 10 µl

Heat samples at **100°C** for 10 minutes before loading.

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2. For Native PAGE

The ExpressPlus™ PAGE Gels are precast without SDS which is conducive for native PAGE. Protein samples should be prepared in non-reducing, non-denaturing sample buffer, to maintain the proteins' secondary structure and native charge. The mobility of the protein depends on the size and shape of the protein as well as its net charge.

Sample preparation

5x sample buffer:

Glycerol	5 ml
Bromophenol Blue	25 mg
Tris base	150 mg
2-Mercaptoethanol	1.0 ml(if necessary)
Deionized water (use 8 M NaOH or 8 M HCl to adjust the pH to 6.8)	to 10 ml

10× MES running buffer:

Tris base	60.6 g
MES	97.6g
EDTA	3g
Deionized water (use 8 M NaOH or 8 M HCl adjust the pH to 7.3)	to 1000 ml

10x MOPS running buffer:

Tris base	60.6 g
MOPS	104.6g
EDTA	3g
Deionized water	to 1000 ml

Note: GenScript's MOPS Running Buffer Powder (Cat No. M00138) contains SDS and is **NOT** suitable for native PAGE.

1x protein sample buffer:

Sample	x µl
Sample buffer (5x)	2 µl
Deionized water	to 10 µl

Do NOT heat the sample.

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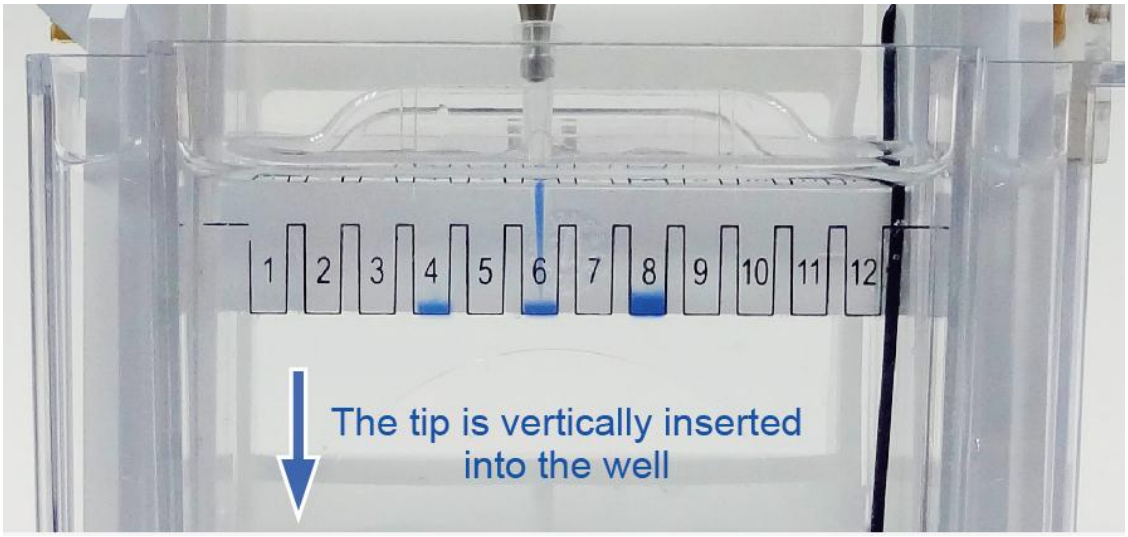
3. Running the sample

   Protein sample loading.

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

Figure 4. Instructions for loading samples



The optimal sample size must be established by trial and error. Protein overloading will cause smearing and distortion. Excessive loading of proteins with free carbohydrates may also lead to band distortion or failure of the protein to penetrate the gel (See Troubleshooting).

Place the rig cover on the gel rig and plug the leads into the power supply (red to red and black to black). Run the gel at 180 volts for 50-55 minutes until color strip reaches the bottom of the gel, users also need to consider the sizes of the proteins of interest (Table 1-3).

Table 3. Electrophoresis conditions for ONE 4-20% ExpressPlus™ 10×10 PAGE Gel

Voltage	**Start**	**Finish**	**Run Time per Gel***
180 V (Recommended)	70-100 mA	30-50 mA	50-55 minutes
200 V	80-110 mA	35-55 mA	45-50 minutes
220 V	90-120 mA	40-60 mA	40-45 minutes
Run gels (recommended at 180V) until the dye front near the bottom of the cassette (about 1cm of the distance). When run gels at higher voltage to obtain shorter run time, the temperature of the running buffer should be controlled, for example, put the gel tank upon the ice, or put an ice bag into the tank chamber.			

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Important notes:

- Make sure to use a compatible gel tank. Leaking between the inner and outer tank will cause slow migration rate, especially when using the Bolt™ Mini Gel Tank. (See Troubleshooting)
- The running time may vary depending on your power supply and the gel concentration.

4. Removing a gel from the Cassette
   a. Once the run is finished, remove the gel from the gel tank according to the manufacturer's instructions.
   b. Open the gel cassette by carefully inserting the cassette opener into the gap between the two plates.
   c. Wiggle the cassette opener up and down gently 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 protective goggles to avoid eye contact or damage.
   d. Upon opening, gel may sit on either side of the cassette. Remove and discard the plate without the gel, and allow the gel to stay on the other plate. Loosen the gel from the plate with water and gently remove. Please dispose of used cassettes as non-hazardous medical waste.

C. Storage

Store ExpressPlus™ PAGE gels flat at 2-8°C. Gels are stable for up to 12 months.

V. STAINING

All standard SDS staining procedures can be used with ExpressPlus™PAGE gels. When using commercially available staining reagents and devices, follow the manufacturer's instructions.

eStain® Staining (Cat No. L02016)

ExpressPlus™ gels can be stained using GenScript's eStain® 2.0 Protein Staining System which allows quick staining of gels in only 7 minutes. See the eStain® 2.0 System manual for staining procedures.
Notes for using eStain® 2.0 Protein Staining System: place the gel horizontally on the staining pad to match the size of the pad.

VI. PROTEIN TANSFER

All standard transferring procedures can be used with ExpressPlus™ PAGE Gels. Using 1x transfer buffer, transfer the proteins at 100 volts for 1 to 2 hours using the wet blotting method. Optimal transfer time must be established by trial and error depending on the sizes of the proteins of interest.

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VII. EXAMPLES

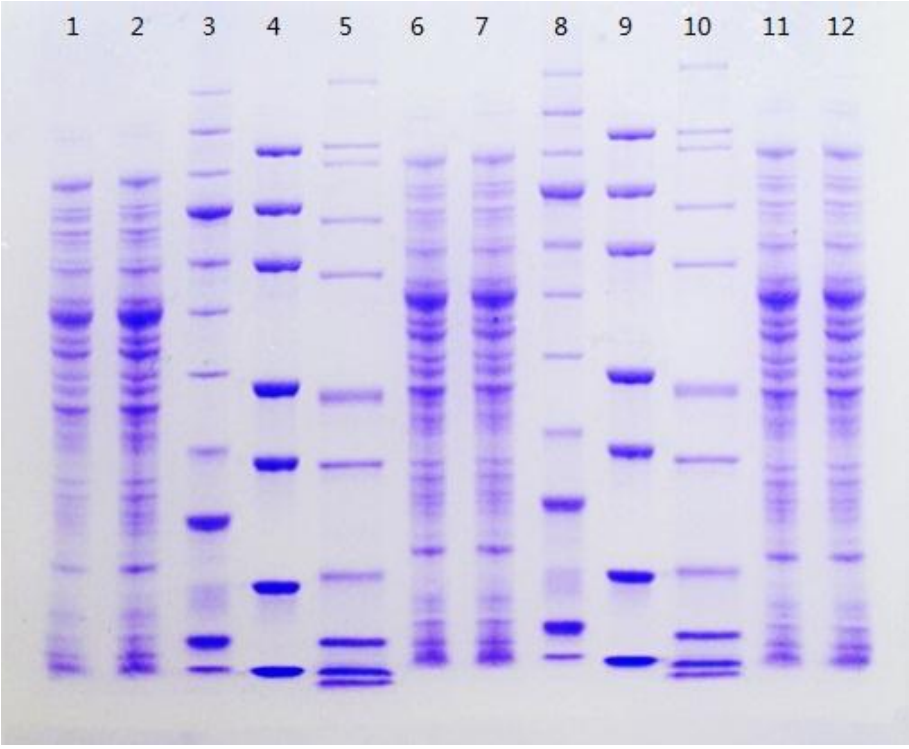

Figure 5. Protein separation using 12% ExpressPlus™ PAGE Gels

Proteins were separated on a 12-well, 12% ExpressPlus™ PAGE Gel and then stained using the eStain® Protein Staining System (R-250).

Lane 1, 2, 6, 7, 11, 12: 6 µl *E.Coli* lysate;
Lane 3, 8: 5 µl NEB Protein Standard (P7703S).
Lane 4, 9: 5 µl GenScript PAGE-Master Protein Standard (M00516);
Lane 5, 10: Life Tech. Mark12™ Unstained Standard (LC5677);

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VIII. TROUBLESHOOTING

Problems	Probable cause	Solution
Distorted protein bands	Air bubbles in the sample wells, or between gel and cassette	Use a syringe or other appropriate tools to flush the sample wells thoroughly with running buffer
Indicator strip partly changed to yellow	Buffer goes into gel through broken cassette	Use compatible gel tanks, make sure the cassette is not cracked
	pH value decreased	Prepare new running buffer with deionized water
Streaking	Poorly soluble 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	Peel the seal at bottom of cassette before loading
	Incorrect running conditions	Use fixed voltage and automated current, eg. 180V throughout the electrophoresis
	Running without a plastic cushion in Bolt™ tank	Put the cushion into the bottom of Bolt™ tank before insert the gel cassette to run
	Running buffer leaking from Bolt™ tank during electrophoresis	Adjust the position of the cassette edge with the gasket of Bolt™ tank to avoid buffer leaking
Bands are difficult to distinguish	Incorrect gel percentage	Use the protein migration table to choose the appropriate gels
	Sample overloading	Reduce sample amount, especially when the sample contains many kinds of protein.
	Insufficient SDS in loading buffer	Enhance SDS in loading buffer when preparing your sample
	Insufficient buffer to keep tank cool	For best results, the buffer in the outer tank should be approximately level with the bottom of the sample wells
Sample spreading across the gel	Sample contains too much salt	Reduce salt by dialysis or ultra-filtration
Ambiguous band at the same position of indicator strip	Ion disturbance in gel (higher chance when analyzing small proteins)	Use MES running buffer
		Run the gel with longer running time or neglect the band
The voltage cannot reach setting value	Leaking between the inner and outer tank during run	Use compatible gel tank

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	Excess salt in the sample	Reduce salt by dialysis or ultra-filtration
Lots of air bubbles between the gel and the cassette	Running buffer is hot after electrophoresis	Run the gel at 4°C
		Increase the running buffer amount in outer tank
The sample volume cannot reach the MAX volume of the sample well	Load the protein sample carefully and slowly	Be careful and slow down for loading

IX. RELATED PRODUCTS AND ORDER INFORMATION

Product	**Cat. No.**
5x Sample Buffer	MB01015
MOPS Running Buffer Powder	M00138
Transfer Buffer Powder	M00139
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
High Range EasyWestern Protein Standard	M00276
eStain® 2.0 Protein Staining Device	L02016
eStain® Protein Staining Pads (R-250, 20-pak)	L02011
eStain® Protein Staining Pads (G-250, 20-pak)	L02012
eStain® Graphite Electrode (11mm, 1-pak)	L02017
PAGE-MASTER Protein Standard (for SDS-PAGE)	M00516
PAGE-MASTER Protein Standard Plus	MM1397-500
WB-MASTER Protein Standard	M00521

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