Version：02
Update：09/30/2022

ExpressPlus™ PAGE Gels, 10×8

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 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 80 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 18 months if stored at 2-8℃
- **Compatible cassette design** – Fits most popular mini-gel tanks
- **High reproducibility** – Guaranteed consistent performance of each gel
- **Cost effective** – Significant reduction in the cost of each experiment

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II. GEL SELECTION GUIDE

Table 1. Gel Selection Guide

Cat.No.	%Acrylamide	Wells	Well Vol.	Running Buffer	Transfer Buffer	Separation Range
M00810	8%	10	80 µl	MOPS, MES	Tris-Bicine	250—15 kDa
M01010	10%	10	80 µl	MOPS, MES	Tris-Bicine	230—10 kDa
M01210	12%	10	80 µl	MOPS, MES	Tris-Bicine	200—6 kDa
M42010	4-20%	10	80 µl	MOPS, MES	Tris-Bicine	250—3.5 kDa
M81610	8-16%	10	80 µl	MOPS, MES	Tris-Bicine	230—6 kDa
M41210	4-12%	10	80 µl	MOPS, MES	Tris-Bicine	250—15 kDa
M00812	8%	12	60 µl	MOPS, MES	Tris-Bicine	250—15 kDa
M01012	10%	12	60 µl	MOPS, MES	Tris-Bicine	230—10 kDa
M01212	12%	12	60 µl	MOPS, MES	Tris-Bicine	200—6 kDa
M42012	4-20%	12	60 µl	MOPS, MES	Tris-Bicine	250—3.5 kDa
M81612	8-16%	12	60 µl	MOPS, MES	Tris-Bicine	230—6 kDa
M41212	4-12%	12	60 µl	MOPS, MES	Tris-Bicine	250—15 kDa
M00815	8%	15	40 µl	MOPS, MES	Tris-Bicine	250—15 kDa
M01115	10%	15	40 µl	MOPS, MES	Tris-Bicine	230—10 kDa
M01215	12%	15	40 µl	MOPS, MES	Tris-Bicine	200—6 kDa
M42015	4-20%	15	40 µl	MOPS, MES	Tris-Bicine	250—3.5 kDa
M81615	8-16%	15	40 µl	MOPS, MES	Tris-Bicine	230—6 kDa
M41215	4-12%	15	40 µl	MOPS, MES	Tris-Bicine	250—15 kDa

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

Table 2. Protein Migration Table

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III. COMPATIBLE GEL TANKS

ExpressPlus™ PAGE Gels are compatible with the following Gel Tanks:

Bio-Rad Mini-PROTEAN® II & 3

Bio-Rad Mini-PROTEAN® Tetra System

LONZA PAGEr® Minigel Chamber

Hoefer Mighty Small (SE 260/SE 250)

Hoefer Tall Mighty Small (SE 280)

Invitrogen Novex XCell I, II, & Surelock® (Use with GenScript Gel Tank Adaptor Plates(Cat.No.L00671))

IV. INSTRUCTIONS FOR USE of ExpressPlus™ PAGE Gels

A. Prepare 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. 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 (see Figure 1).

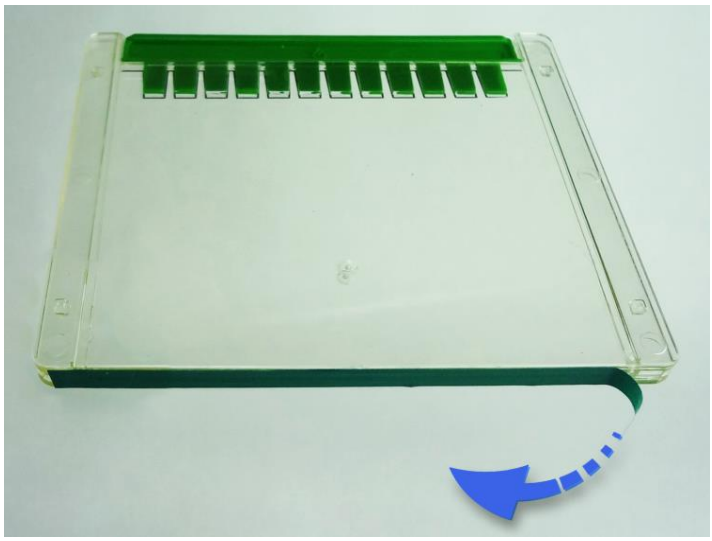

Figure 1. Peel the tape from the bottom of the cassette

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3. Remove the comb from the gel cassette gently (see Figure 2).

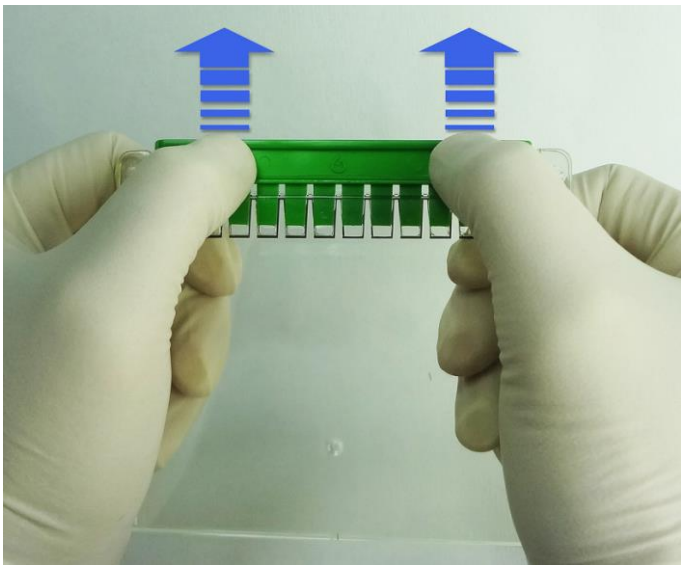

Figure 2. Remove the comb from the gel cassette

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 frame, turn it around so the flat side is facing outwards and insert the gasket back into the inner frame (see Figure 3).

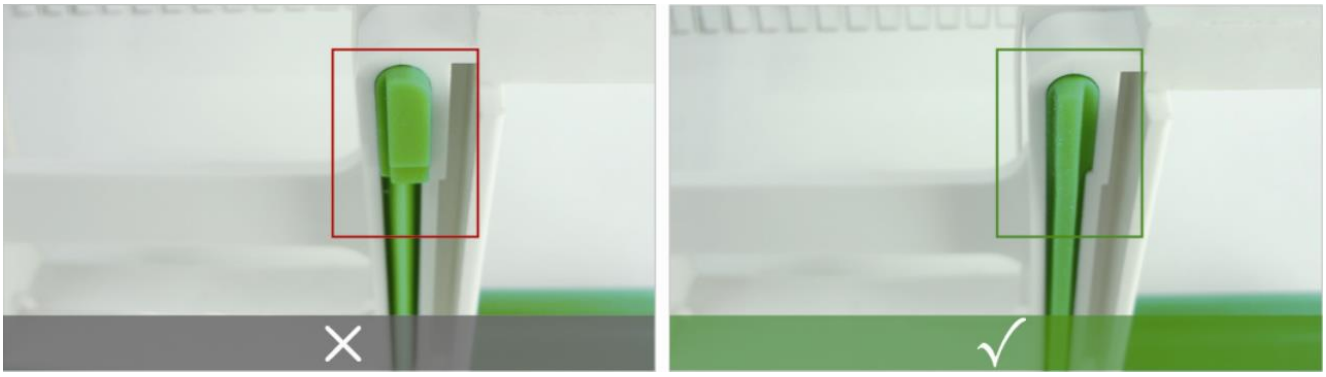

Figure 3. Use of ExpressPlus™ PAGE Gels in Bio-Rad Mini-PROTEAN® Tetra System

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Notes for using Invitrogen Novex Mini-Cell tanks: Tank Adaptor (Cat. No.L00671) are needed since the ExpressPlus™ PAGE Gel cassette is thinner than the Invitrogen NuPAGE® gel cassette, one adaptor is corresponded with one gel, independently.

See figure 4 for use of ExpressPlus™ PAGE Gels in the Invitrogen Novex® Mini-Cell.

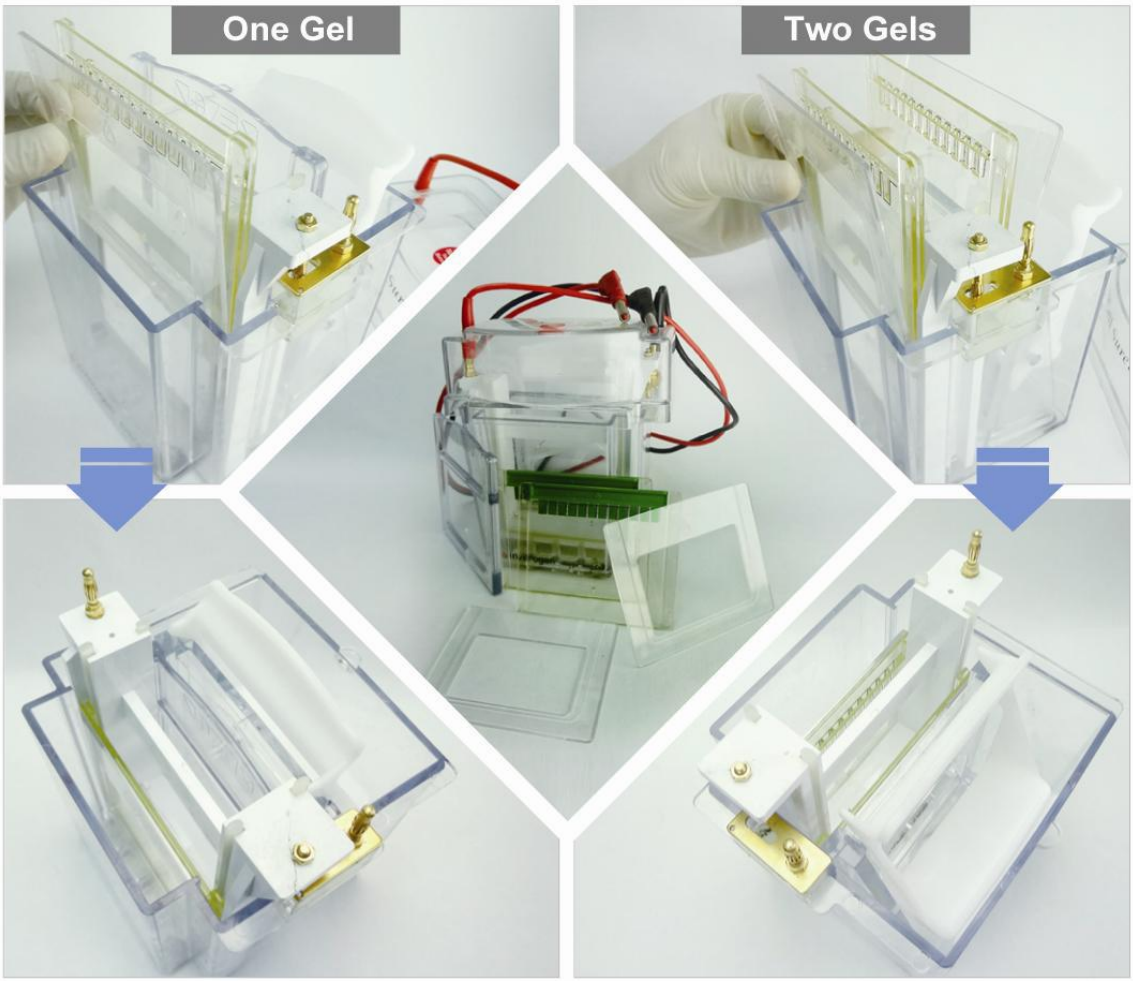

Figure 4. Use of ExpressPlus™ PAGE Gels in Invitrogen Novex® Mini-Cell

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.

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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

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

1x protein sample buffer:

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

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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	5.0 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

1× MES running buffer:

Tris base	6.06 g
MES	9.76g
EDTA	0.3g
Deionized water	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 Tris-MOPS-SDS 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.

3. Running the sample

Protein sample loading.

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

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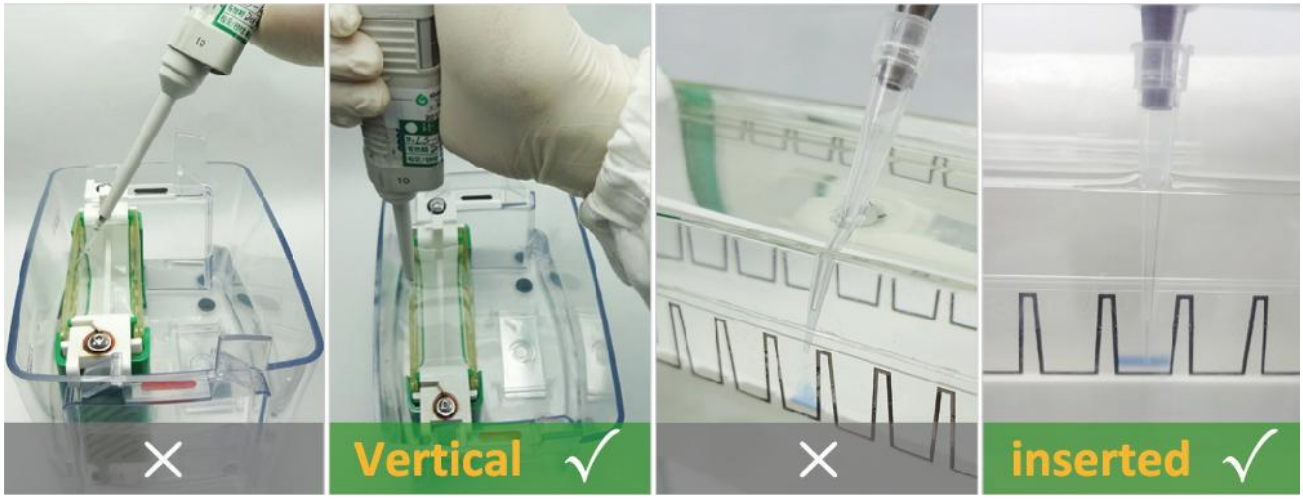

Figure 5. Use of sample loading

Note: 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 140 volts for 45-55 minutes until color strip reaches the bottom of the gel, depending on the sizes of the proteins of interest (Table 3).

Table 3. Electrophoresis conditions for ONE ExpressPlus™ PAGE Gel

Voltage	Start	Finish	Run Time per Gel*
140 V (Recommended)	75-100 mA	30-50 mA	45-55 minutes
*Gel running time depends on the temperature in the laboratory. These run times are recommended at a laboratory temperature of 20°C with Tris-MOPS-SDS buffer.			

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 vary depending on your power supply and the gel concentration.

4. Removing a gel from the Cassette (see Figure 6)
    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

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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.

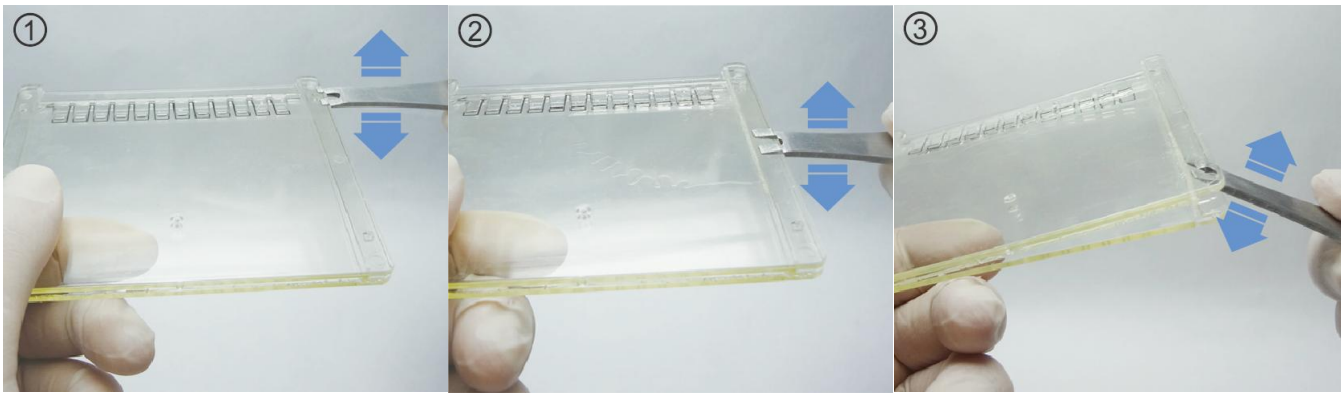

Figure 6. Open the gel cassette to remove the gel.

C. Storage

Gels are stable for up to 18 months if stored at 2-8°C.

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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.

Coomassie Staining - for homemade Comassie R-250 staining**:**

1) Staining solution: 0.1% (m/v) Coomassie R-250, 40% ethanol and 10% acetic acid solution in deionized water.
2) Destaining solution: 10% ethanol and 7.5% acetic acid solution in deionized water.
3) Open the gel cassette and take the gel out gently after electrophoresis, then put the gel in a staining container of 100ml stainning solution.
4) Cover the staining container and heat in a microwave oven at full power for 8 minutes. To prevent hazardous, flammable vapors from forming, do **NOT** allow the solution to boil.
5) Remove the staining container from the microwave oven and gently shake the gel for 5 minutes at room temperature on an orbital shaker.
6) Drain the staining solution and rinse the gel with deionized water.
7) Place the stained gel in a staining container of 100 ml destaining solution.
8) Cover the staining container and heat in a microwave oven at full power for 8 minutes.
9) Drain the destaining solution, add fresh destaining solution, repeat step 8.
10) Gently shake the gel at room temperature on an orbital shaker until the desired background is achieved.

eStain™ L1 Protein Staining Device (Cat. No. L00657)

ExpressPlus™ gels can be stained using GenScript's eStain™ L1 Protein Staining Device which allows quick staining of gels in only 10 minutes. See the eStain™ L1 Protein Staining Device manual for staining procedures.

VI. PROTEIN TRANSFER

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

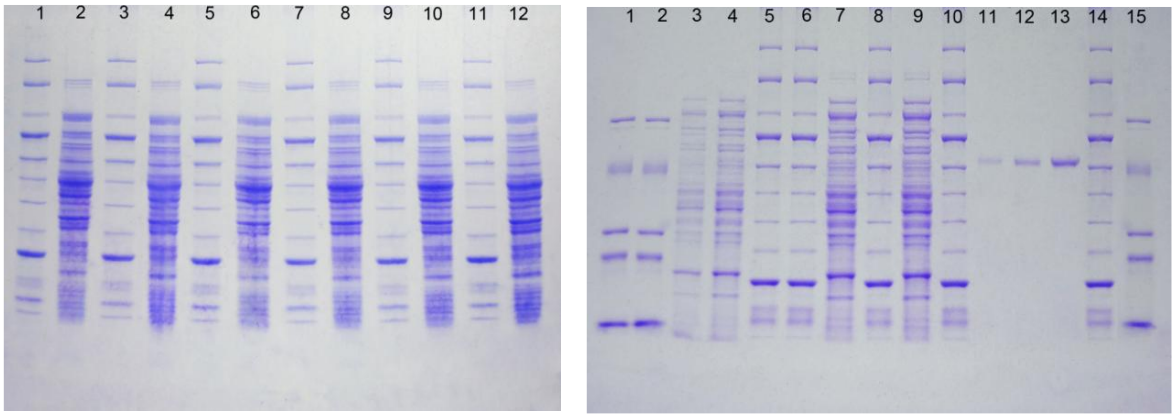

Figure 7. Protein separation using 4-20% ExpressPlus™ PAGE Gels

Proteins were separated on a 12-well, 4-20% ExpressPlus™ PAGE Gel and a 15-well, 4-20% ExpressPlus™ PAGE Gel and then stained using the eStain™ Protein Staining System (R-250).

(L):

Lane 1, 3, 5, 7, 9, 11: 8 μl New England Biolabs® 10-250 kDa protein ladder (P7703S);

Lane 2, 4, 6, 8, 10, 12: 7 μl *E.coli* cell lysate.

(R):

Lane 1, 2, 15: GenScript Broad Protein Marker II (M00505);

Lane 3, 4, 7, 9: 5 μl *E.coli* cell lysate;

Lane 5, 6, 8, 10, 14: 6 μl New England Biolabs® 10-250 kDa protein ladder (P7703S);

Lane 11, 12, 13: 150 ng/ 300 ng/ 600 ng BSA.

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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. 140V throughout the electrophoresis
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
	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

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IX. RELATED PRODUCTS AND ORDER INFORMATION

Product	Cat. No.
5x Sample Buffer	MB01015
4X LDS Sample Buffer	M00676
Tris-MOPS-SDS Running Buffer Powder	M00138
Transfer Buffer Powder	M00139
eStain™ L1 Protein Staining Device	L00657
PAGE-MASTER Protein Standard (for SDS-PAGE)	M00516
PAGE-MASTER Protein Standard Plus	MM1397-500
WB-MASTER Protein Standard	M00521
Broad Multi Color Pre-Stained Protein Standard	M00624
eBlot™ L1 Protein Transfer System	L00686
Cassette opener	L00674
Buffer Dam	L00699
Tank Adaptor (for use with Novex gel tanks)	L00671

For research and manufacturing use. Direct human use, including taking orally and injection are forbidden.

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