Express PAGE Gels **Technical Manual No. 0210**

I. INTRODUCTION

GenScript Express PAGE Gels are high-performance precast mini polyacrylamide gels for protein electrophoresis that exhibit long shelf live, fast run time and efficient transfer. Express Gels are cast in a neutral pH buffer that minimizes the hydrolysis of polyacrylamide and results in extra gel stability and superior band resolution.

Manufactured without SDS, Express 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 reproducibility and guarantee the reliable migration pattern. Using specially formulated Tris-MOPS running buffer, Express Gels enable proteins to be separated quickly and easily for subsequent detection by staining or Western blotting.

The Express Gels are available in gradient (4 to 20% and 8 to 16%) and fixed (8%, 10% and 12%) concentrations and in 10-well, 12-well and 15-well formats.

Key Features:

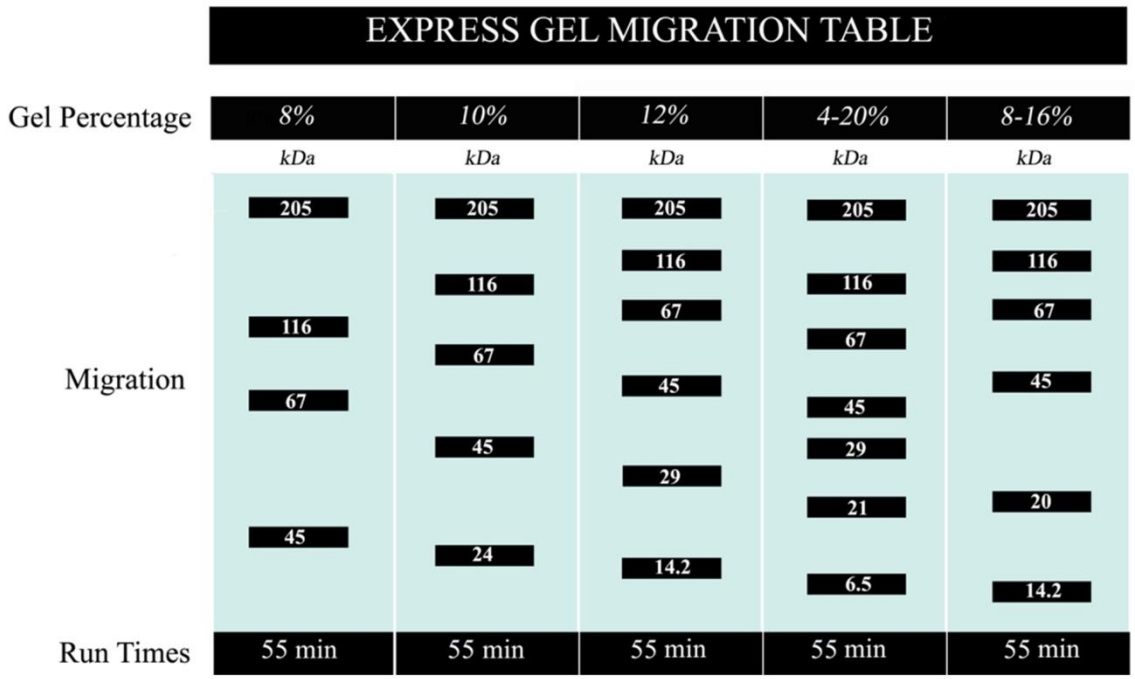
- **Easy to use** –simple to set up, no comb or tapes to be removed.
- **High resolution** – unique running buffer providing excellent separation and high-resolution bands.
- **Long shelf life** – up to 12 months at 4°C
- **Compatible cassette design** – fit most of popular mini-gel tanks
- **High reproducibility** – guaranteed consistent performance of each piece of gel.
- **Cost effective** – significant reduction in the cost of each experiment.

II. GEL SELECTION GUIDE

Table 1. Gel Selection Guide

Cat. No.	% Acrylamide	Wells	Well Vol.	Running Buffer	Transfer Buffer	Separation Range
MG008W10	8%	10	50µl	Tris-MOPS-SDS	Tris-Bicine	205 - 45 kDa
MG010W10	10%	10	50µl	Tris-MOPS-SDS	Tris-Bicine	205 - 24 kDa
MG012W10	12%	10	50µl	Tris-MOPS-SDS	Tris-Bicine	205 - 14 kDa
MG420W10	4-20%	10	50µl	Tris-MOPS-SDS	Tris-Bicine	205 – 6.5 kDa
MG816W10	8-16%	10	50µl	Tris-MOPS-SDS	Tris-Bicine	205 - 14 kDa
MG008W12	8%	12	30µl	Tris-MOPS-SDS	Tris-Bicine	205 - 45 kDa
MG010W12	10%	12	30µl	Tris-MOPS-SDS	Tris-Bicine	205 - 24 kDa
MG012W12	12%	12	30µl	Tris-MOPS-SDS	Tris-Bicine	205 - 14 kDa
MG420W12	4-20%	12	30µl	Tris-MOPS-SDS	Tris-Bicine	205 – 6.5 kDa
MG816W12	8-16%	12	30µl	Tris-MOPS-SDS	Tris-Bicine	205 - 14 kDa
MG008W15	8%	15	25µl	Tris-MOPS-SDS	Tris-Bicine	205 - 45 kDa
MG010W15	10%	15	25µl	Tris-MOPS-SDS	Tris-Bicine	205 - 24 kDa
MG012W15	12%	15	25µl	Tris-MOPS-SDS	Tris-Bicine	205 - 14 kDa
MG420W15	4-20%	15	25µl	Tris-MOPS-SDS	Tris-Bicine	205 – 6.5 kDa
MG816W15	8-16%	15	25µl	Tris-MOPS-SDS	Tris-Bicine	205 - 14 kDa

This protein migration table can also help you with choosing the appropriate gels for your protein electrophoresis analysis.



III. COMPATIBLE GEL TANKS

Express PAGE Gels are compatible with the following Gel Tanks:

- GradiGel Mini 4-Cell
- IBI Universal Protein System
- EC 4-Cell
- Hoefer Tall Mighty Small™ (SE 280)
- Hoefer Mighty Small™(SE 260/SE 250)
- Daiichi Mini 2-Gel & 6-Gel
- Owl Road Runner, Penguin
- Bio-Rad Mini-PROTEAN™ Tetra cell
- Owl Single Sided Vertical System

IV. INSTRUCTIONS FOR USE OF EXPRESS PAGE GELS

A. Prepare Gel Buffer and Gel Tank

1. Dissolve one pack of MOPS running buffer powder (Product No. M00138) in 1L ultrapure water to make 1L 1 X running buffer, which is sufficient for one electrophoresis unit.
2. Remove Express PAGE Gel from the pouch and insert into the gel running apparatus (see Figure 1). Refer to the apparatus manufacturer's instructions.

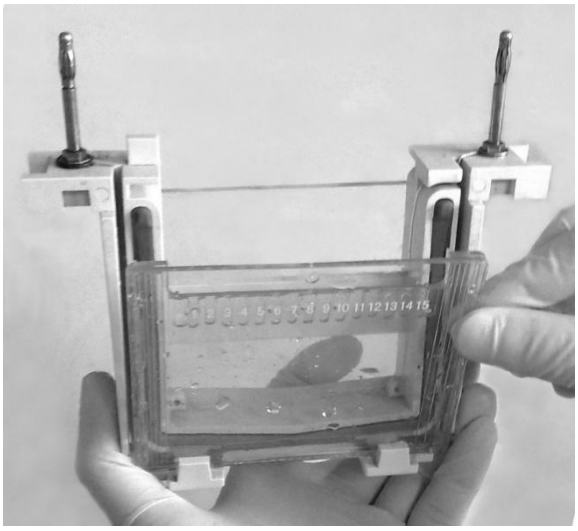


Figure 1. Inset the Express Gel into cassette

3. Pour sufficient 1X 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 1X 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
4. Rinse the sample wells thoroughly with 1X MOPS running buffer with a pipette to remove air bubbles and displace any storage buffer.

Notes for Using Bio-Rad Mini-PROTEAN Cell Tanks: remove the gasket from the inner frame (see Figure 2), turn it around so the flat side is facing outwards and re-insert into the inner frame.

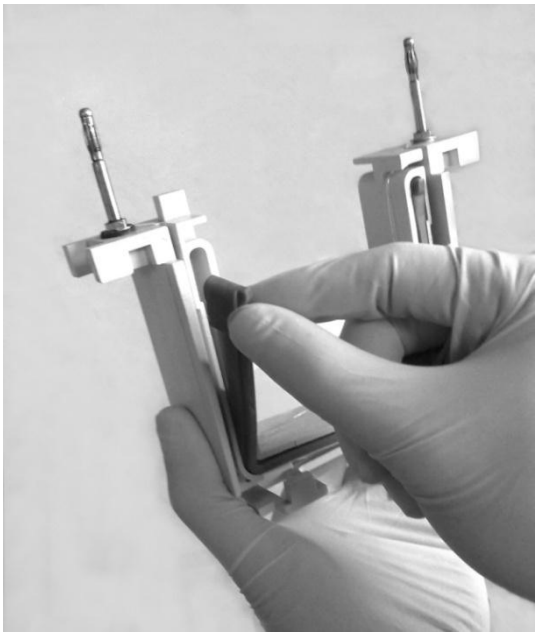


Figure 2. Turn around the gasket when using Bio-Rad Mini-PROTEAN Cell Tanks

B. Sample Preparation

1. For SDS PAGE Gel

SDS Sample preparation

Recipe of 5×sample buffer:

SDS	1.0 g
Glycerol	5.0 ml
Bromophenol Blue	25 mg
Tris base	150 mg
2-Mercapoethanol	1.0 ml
Deionized water to	10 ml
(adjust the pH to 6.8)	

Recipe of 10× Running Buffer:

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

Recipe of 1× protein sample buffer:

Sample	x µl
GenScript's sample buffer (5×)	2 µl
Deionized water	to 10 µl

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

2. For Native PAGE

The Express PAGE Gels are precast without SDS and allow the native PAGE. Protein samples are prepared in non-reducing and non-denaturing sample buffer, which maintains the proteins' secondary structure and native charge. The mobility of protein depends on the size and shape of the protein as well as its net charge.

Sample preparation

Recipe of 5×sample buffer:

Glycerol	5.0ml
Bromophenol Blue	25mg
Tris base	150mg
2-Mercapoethanol	1.0ml (if necessary)
Deionized water to (adjust the pH to 6.8)	10ml

Recipe of 10× Running Buffer:

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

Note: GenScript's Tris-MOPS-SDS running buffer powder (Cat No. M00138) contains SDS and is NOT suitable for native PAGE.

Recipe of 1×protein sample buffer:

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

Don't heat the sample.

C. Sample Running

1. Apply protein sample (in 1X Sample Buffer and incubated at 70^{o}C for 10 minutes) up to 50 µg (total protein) per well. Optimal sample size must be established by trials. Overloading will cause smearing and distortion. Excessive loading of proteins with free carbohydrate may also lead to band distortion or failure of the protein to penetrate into the gel (See 'Trouble Shooting').

2. Put the rig cover onto 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 55 minutes depending on the sizes of proteins of interest (Table 2).

Table 2. Electrophoresis conditions for ONE piece of Express PAGE Gel			
Approximate Current			
Voltage	**Start**	**Finish**	**Run Time per Gel***
140 V	100-125 mA	60-80 mA	~55 minutes
*Gel running time depends on the temperature in the laboratory. These run times are recommended at a laboratory temperature of 20°C.			

Important notes:

- ◇ Make sure using the compatible gel tank. Leaking between inner and outer tank will lead to low voltage and low migrate rate. (see Troubleshooting)
- ◇ The running time may be varied depending on your samples.

D. 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. Insert a coin or a small metal spatula in one of the slots at the bottom of the gel cassette and twist to open the gel cassette. Pull the top plate of the cassette away from the bottom plate. The two halves will snap apart completely, exposing the gel (Figure 3).
3. Loosen the gel at the bottom with water and remove.

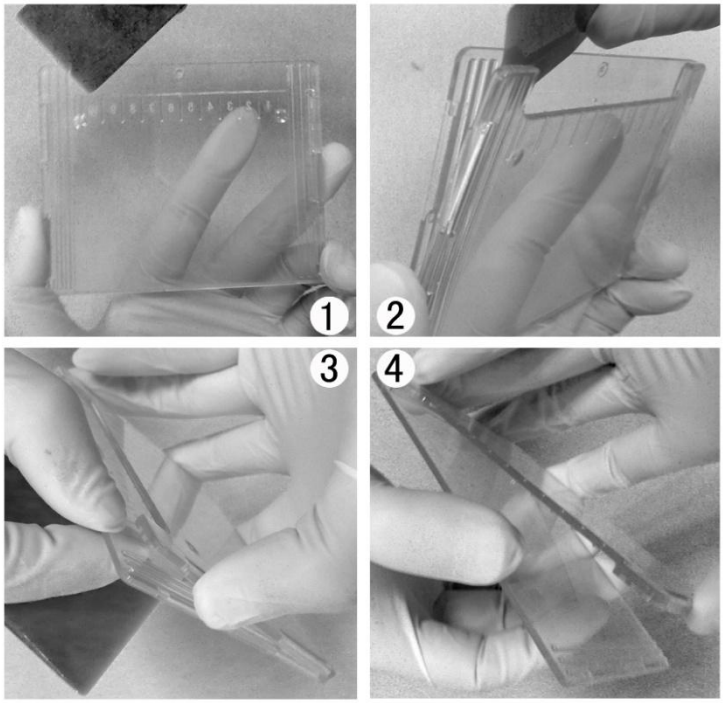


Figure 3. Opening the cassette to expose the gel.

V. STAINING AND DRYING GELS

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

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

- 0.1% Coomassie R-250 in 40% ethanol and 10% acetic acid for staining solution.
- 10% ethanol and 7.5% acetic acid for destaining solution.
- Staining procedures can be referred to related instructions.

eStain™ Staining (Cat No. L02010)

Express gels can be stained using GenScript's eStain™ Protein Staining System which allows quick staining of gels within only 7 minutes. See the eStain™ System manual for the detailed staining procedures.

Gel drying

Express PAGE Gels can be dried by passive evaporation (air-drying), vacuum drying or other gel drying methods.

VI. PROTEIN TANSFER

All the standard transferring procedures can be used with Express PAGE Gels. Using 1X transfer buffer, transfer the blot at 40 volts for 90 minutes using Wet Blotting method or transfer the blot at 25 volts for 30 minutes using Semi-Dry Blotting method. Optimal transfer time must be established by trials depending on the sizes of proteins of interest.

VII. EXAMPLES

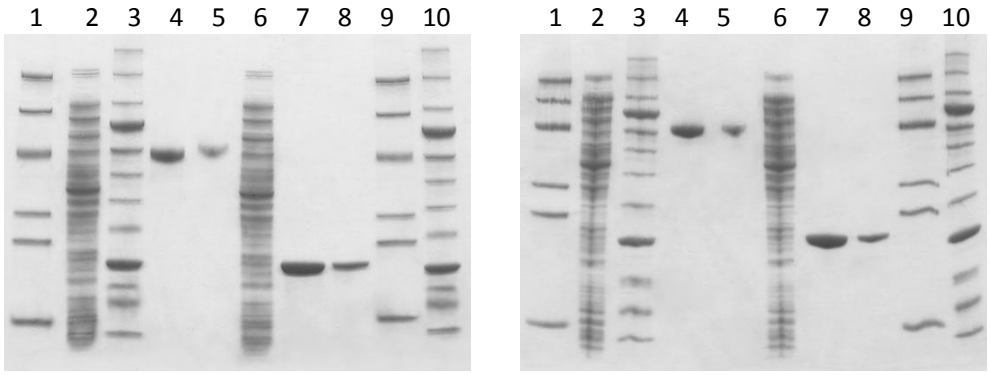


Figure 4. Comparison of protein separation with Express PAGE Gels and Invitrogen's precast gel. Proteins were separated on a 12% Express PAGE Gel (L) and a 12% Bis-Tris gel from Invitrogen (R) and then stained using eStain™ Protein Staining System (R-250). Lane 1 and 9: 10µl Genscript 14-150 kDa protein ladder; Lane 2 and 6: 10µl *E.coli* BL21 cell lysate; Lane 3 and 10: 10µl New England Biolab® 10-250 KDa protein ladder (P7703S); Lane 4 and 5: 500 ng and 100 ng BSA; Lane 7 and 8: 500 ng and 100 ng GST protein.

VIII. TROUBLESHOOTING

Problems	Probable cause	Solution
Distorted protein bands	Air bubbles in the sample wells, or between gel and cassette.	Use a transfer pipette or syringe to flush the sample wells thoroughly with running buffer.
streaking	Poorly soluble or weakly charged particles (such as carbohydrates) in sample.	Heat sample in the presence of SDS, centrifuge sample and use the supernatant.
Bands difficult to distinguish	Incorrect gel percentage.	Use the protein migration table to help you choose the appropriate gels for your protein electrophoresis.
	Sample overloading.	Reduce sample size, do not load more than 50 µg (total protein) per well.
	Insufficient buffer to cool down the gel.	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.
The dye front partly change to yellow	Running buffer is bad.	Make your MOPS running buffer with deionized water again.
	The running buffer goes into the gel because the cassette is curved by using unsuitable tank.	Use compatible gel tank, make sure the gel cassette is not too tight to insert into the tank.
The voltage cannot reach setting	Leaking between the inner and outer tank when running.	Use compatible gel tank
	Excess salt in the sample.	Reduce salt by dialysis or ultra-filtration.
Lots of air bubble between the gel and the cassette	Running buffer is hot after electrophoresis.	Don’t keep the room temperature very high.
		Increase the running buffer in outer tank

IX. RELATED PRODUCTS AND ORDER INFORMATION

Product	Cat. No.
5×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
Graphite Electrode	