Express™ PAGE Gels

Technical Manual No. 0210 **Update date 10112010**

I. DESCRIPTION

GenScript's Express™ PAGE Gels are precast polyacrylamide gels for protein gel electrophoresis. Using proprietary techniques, Express™ PAGE Gels are made to have long shelf life, high resolution, fast electrophoresis, and high transfer efficiency. Express™ PAGE Gels are better choices for protein analysis with the following major breakthroughs:

Express™ PAGE Gels are better choices for protein analysis with the following major breakthroughs:

- **Easy to use** – no comb or tapes to be removed, very simple to set up.
- **Long shelf life** – one year (stored at 4°C).
- **High resolution** – using unique running buffer to produce excellent separation and high-resolution bands.
- **Fast electrophoresis** – 30 to 45 minutes run time for quick results.
- **High transfer efficiency** – complete protein transfer in 30 min using semi-dry technique.

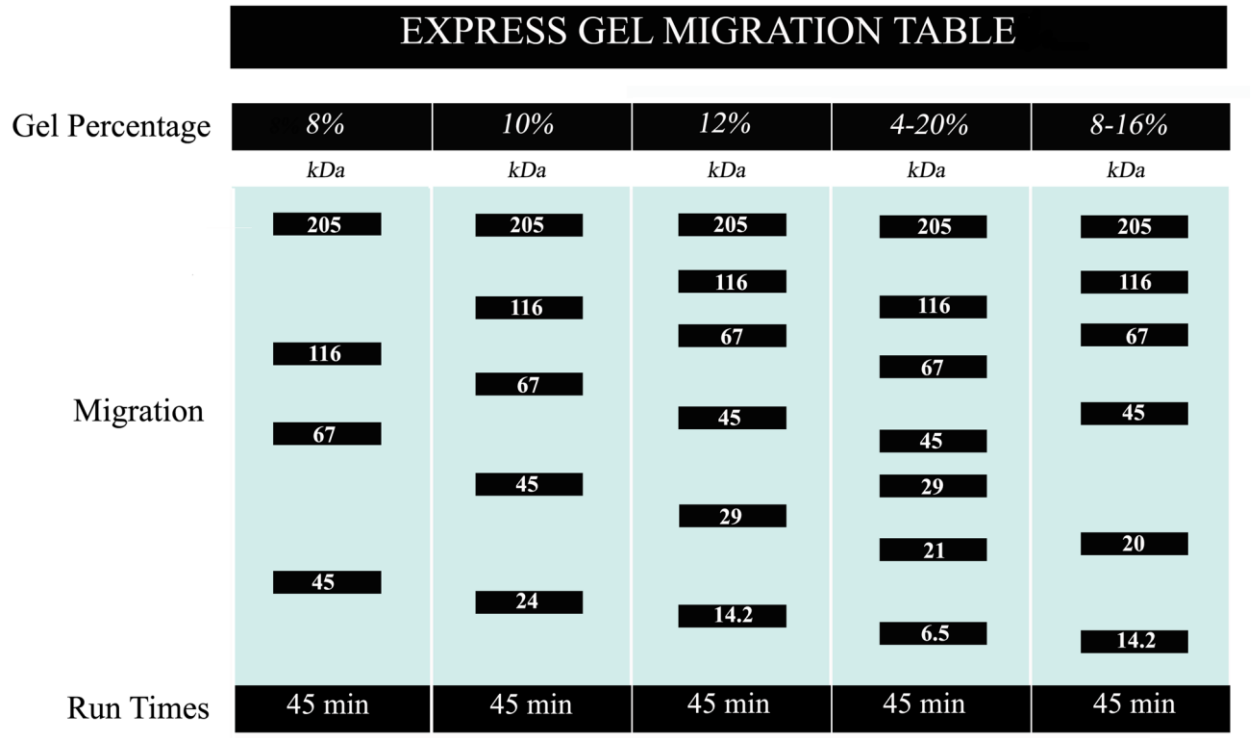
II. GEL SELECTION GUIDE

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

MG816W12	8-16%	12	30 µl	Tris-MOPS-SDS	Tris-Bicine	205 - 14 kDa
MG008W15	8%	15	25 µl	Tris-MOPS-SDS	Tris-Bicine	205 - 45 kDa
MG010W15	10%	15	25 µl	Tris-MOPS-SDS	Tris-Bicine	205 - 24 kDa
MG012W15	12%	15	25 µl	Tris-MOPS-SDS	Tris-Bicine	205 - 14 kDa
MG420W15	4-20%	15	25 µl	Tris-MOPS-SDS	Tris-Bicine	205 - 6.5 kDa
MG816W15	8-16%	15	25 µl	Tris-MOPS-SDS	Tris-Bicine	205 - 14 kDa

III. PROTEIN MIGRATION TABLE

This protein migration table can also help you to choose the appropriate gels for your protein electrophoresis. The data of this table is from gels run at 140 volts for 45 min. At 140 volts, the Bromophenol Blue band will reach the bottom of the gels in about 30 min.



IV. COMPATIBLE GEL TANKS

Express 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
- Novex XCell I and II™ and Surelock™
- Bio-Rad Mini-PROTEAN™ II & 3
- Owl Single Sided Vertical System

V. RELATED PRODUCTS

Product	Cat. No.	Size
5X Sample Buffer	MB01015	5 ml
Express Gel Running Buffer Powder*	M00138	To make 1000ml 1X Running Buffer
Transfer Bufffer Powder*	M00139	To make 1000ml 1X Transfer Buffer
Novel Coomassie brilliant blue stain	C01714	250 ml

Recipe of 5X Sample Buffer:

5X Sample Buffer:		
SDS	1.0 g	
Glycerol	5.0 ml	
Bromophenol Blue	25 mg	
Tris base	150 mg	(adjust the pH to 6.8)
2-Mercapoethanol	1.0 ml	
Deionized water to	10 ml	

Recipe of 10X Running Buffer and 20X Transfer Buffer:

	10X Running Buffer	**20X Transfer Buffer***
Tris base	60.6g	60.0 g
Bicine		81.6 g
MOPS	104.6g	
SDS	10.0 g	
EDTA	3.0 g	
Deionized water to	1000 ml	1000 ml

*To make 1L of 1X transfer buffer: mix 50 ml of 20X Transfer Buffer, 100 ml of methanol or ethanol and 850 ml of deionized water.

VI. STORAGE

Store all the Gels and Buffers at 4°C - 8°C.

VII. INSTRUCTIONS FOR USE OF EXPRESS™ PAGE GELS

1. Remove gel from packet and insert into the gel running apparatus. Refer to the apparatus' manufacturer's instructions*.
2. 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.
3. Using a transfer (pasteur) pipette or syringe, flush the sample wells thoroughly with running buffer to remove air bubbles and to displace any storage buffer.
4. Apply protein sample (in 1X Sample Buffer and incubated at 100^{o}C for 5 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').
5. 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 30 to 45 minutes depending on the sizes of proteins of interest.
6. Once you have finished the run, remove the gel from the gel tank according to the manufacturer's instructions. 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. Remove the gel and proceed with staining or transferring.
   A. Stainning. All the standard staining procedures can be used with Express™ PAGE Gels.
   B. Transferring. 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.

***More instructions for using different gel running apparatus:**

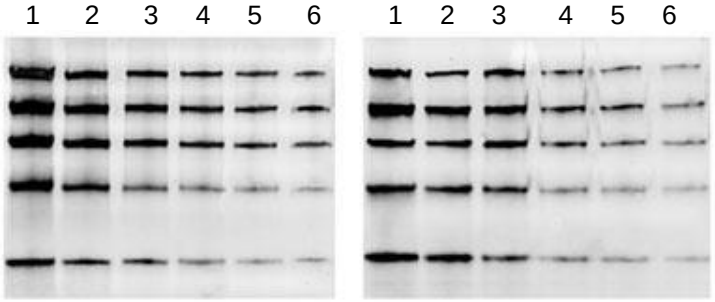
In each box of gels, one plastic adaptor plates are included to facilitate gel set-up. For example, if you are using Bio-Rad Ready Gel Cell to run one gel, you can put this plastic adaptor plates at the other side of the gel rig to form a tight seal to prevent current leakage. Also, when you are using Bio-Rad Ready Gel Cell (or Bio-Rad
Mini Protean), you need to remove the plastic gasket (from both sides) from the inner frame, turn the gasket around, so that the flat side is facing outwards and re-insert the gasket into the inner frame.

When you are using NOVEX tank to run one gel, put the gel at the side of the gel rig away from the wedge devices, and put two of these plastic adaptor plates at the other side of the gel rig close to the wedge devices. If you are using NOVEX tank to run two gels, put one gel at the side of the gel rig away from the wedge devices, and put the second gel and one plate at the other side of the gel rig close to the wedge devices. The second gel should be inside and face the first gel.

VIII. EXAMPLES USING THE GELS

Comparison of Express™ Precast Gels with other Precast Gels.

Express™ PAGE Gels were compared with precast gels (Brand A) from company A for Western Blot detection of His-tagged proteins.



Express™ Gels Company A. precast gels

	Express™ Precast Gel	Brand A Precast Gel
Gel Concentration	**4 - 20%**	**4 - 20%**
Gel Running	140 volts **45 min**	140 volts **45 min**
Semi-Dry Protein Transfer	20 volts **30 min**	30 volts **45 min**

Figure 1. Comparison of Express™ Precast Gels with other Precast Gels for Western Blot detection of EasyWestern Protein Marker. 10 µl, 5.0 µl, 2.5 µl, 1.3 µl , 0.6µl,0.3µl of the EasyWestern Protein Marker (Genscript, Cat. No.MM0908), were loaded in Lane 1, Lane 2, Lane 3, Lane 4,Lane 5 and Lane 6 of a 4 – 20% Express™ PAGE Gel, respectively. After electrophoresis, the EasyWestern Protein Marker were transferred to nitrocellulose membrane and then detected using One-Step™ His-Tag Western Detection Kit L00212.

This experiment was repeated by using a 4 – 20% Precast Gel (Brand A) from company A.

Comapred with Other Product , Express Gels needed less time and gave better results, bigger proteins (in this case, 75 kD and 100 kD His-tagged proteins) were transferred more efficiently from Express Gels.

IX. TROUBLESHOOTING

The table below is only a guideline for troubleshooting.

Problem	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.

X. ORDER INFORMATION

See the “gel selection guide”.

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