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User Guide

AccuPAGE™

Precast PAGE Gel Bis-Tris & Tris-Glycine

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Introduction

Bio Bench AccuPAGE™ protein electrophoresis gels are precast polyacrylamide gels that provide large range of molecular weight separation ability with good resolution and sample integrity. AccuPAGE™ provides 2 types of gels, including conventional **Tris-Glycine gel** and the **Bis-Tris gel**. These 2 types of gel are both optimized by Bio Bench, in order to provide better performance as well as good stability during long term storage. AccuPAGE™ is compatible with nearly all of tanks from major biotech providers. Take out from the fridge and open the package, then you can operate it under the routine lab instructions.

AccuPAGE™ Tris-Gly gel is made of optimized formula from conventional Tris-Glycine gel. It has good stability and long shelf life for up to 12 months. If you lab has been using Tris-Glycine gel, then you can shift to **AccuPAGE™ Tris-Gly precast gel** smooth.

AccuPAGE™ Bis-Tris gel delivers consistent gel performance with a neutral pH environment (pH≈7.0) that minimizes any protein modifications. Bis-Tris can improve the stability of the gel structure during electrophoresis, which will avoid the gel become trapezoid shape and lead to sharp and straight band after staining. Your lab device does not need to be changed to run **AccuPAGE™ Bis-Tris gel** – only need to remember Bis-Tris gel need to be run in MOPS or MES buffer.

Key advantages:

1. Large range of protein size compatibility from 3.5kDa to 300kDa. Precast Gels Plus are available in different acrylamide concentrations, including 6%, 8%,10%,12% and 4-12%, 4-15% and 4-20%. Customized services for special concentrations are also available.
2. Good compatibility with electrophoresis tanks from almost all brands, including but not limited to: Bio-Rad, Life Technology (Thermo), Hoefer, Sigma Aldrich Dual Run, Thermo XCell I, II, & Surelock™ mini-cell, LONZA PAGEr® Minigel Chamber and Tanon, etc.
3. Tested in-house by Bio Bench's CRO services.
4. Automated gel casting platform ensures stability and repeatability of the results.
5. Coated plastic cassette which is able to avoid protein absorption and ensures the clear and sharp protein band.
6. Rapid electrophoresis within 25min by Ultra Fast Running Buffer.
7. Compatible with all types of protein marker, including conventional marker and pre-stained marker.
8. Gel can be observed by Coomassie brilliant blue staining, silver staining, fluorescent staining or under UV light.
9. Gel cassettes can be easily opened with the common Gel Opener.
10. AccuPAGE™ Precast Gels is free of any detergent (like SDS). User can run both native PAGE or denaturing PAGE.

Specification of the AccuPAGE™ Precast Gel

Specifications	Tris-Glycine Gel	Bis-Tris Gel
Concentration	6%, 8%, 10%, 12%, 15%, 4-15%, 4-20%	8%, 10%, 12%, 4-12%
Gel Dimension	Mini gel, 84mm(W)×74mm(H) ×1mm(D)	
Cassette Dimension	100mm(W)×89mm(H) ×4.8mm(D)	
Gel Thickness	1mm	
Stacking Gel	Concentration 4%, 15mm(H)	
Well amount and max load	10 wells 50µL, or 15 wells 30µL.	
Storage and Shelf Life	4 - 8°C, up to 12 months. DO NOT FREEZE.	4 - 8°C, up to 12 months. DO NOT FREEZE.
Shipping	Room temperature or controlled temperature.	
Tank Compatibility	AccuPAGE™ is designed to be compatible with all types of MINI-PAGE tank which allows 10cm width gel cassette. Including but not limited to the following models: 1. Bio-Rad Mini-PROTEAN (II/3 /Tetra System) * 2. Hoefer™ Mighty Small (SE250 / SE260 / SE280) 3. Sigma Aldrich Dual Run * 4. Thermofisher/Invitrogen XCell (I, II, & Surelock™ mini-cell) * 5. LONZA PAGEr® Minigel Chamber * 6. Tanon	
Running Time	Minimum 20min if Ultra Fast Running Buffer is used. 40 – 60 min by using common buffer.	
Application	Native PAGE, denaturing PAGE. Note: Gel does NOT contain detergent (like SDS).	
Running Buffer	Tris-Glycine	MOPS or MES
Package Quantity	2 pcs test pack, 10 pcs, 30 pcs and 50 pcs.	

* Accessory or adaption will be needed. Bio Bench provides the accessories.

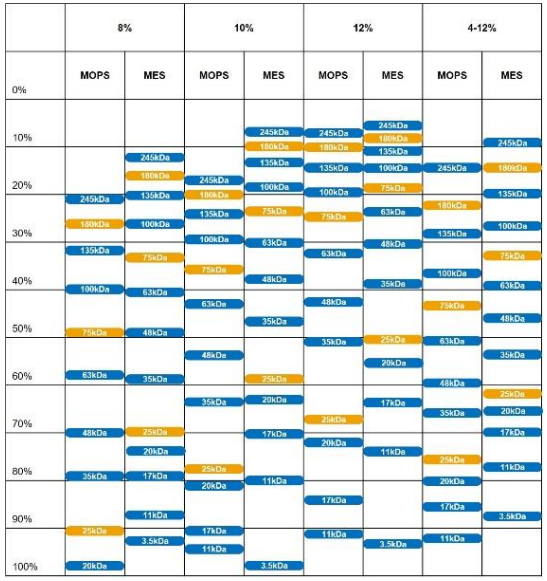
Storage and Stability

AccuPAGE™ Precast Gels have extended shelf-life of up to 12 months from the date of production when stored at 4–8 °C.

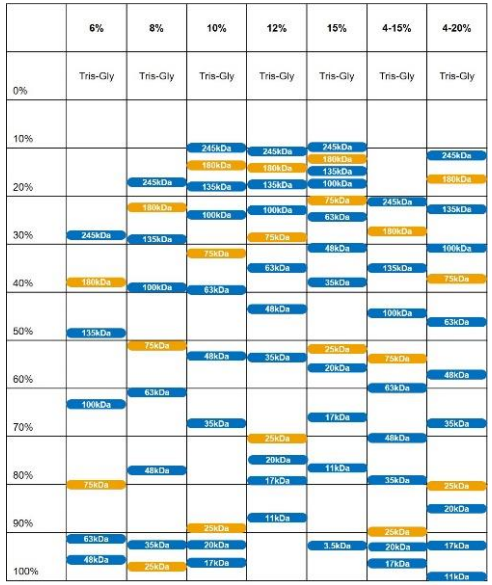
AccuPAGE™ Precast Gels shall be laid flat during the storage. “UP” arrow shows the direction of the box during storage.

Protein Separation using AccuPAGE™ Precast Gels

The chart under is showing the relative migration of the protein in denatured condition. It will help you to select the gel.



AccuPAGE™ Bis-Tris Precast Gel, denatured condition



AccuPAGE™ Tris-Glycine Precast Gel, denatured condition

AccuPAGE™ Precast Gel List and Accessories

Gel type	Running Buffer	Gel Thickness	Gel Concentration	Max Separation	Recommended Separation	Well Amount	Max Sample Load	Cat. No.
Tris-Glycine	Tris-Glycine	1mm	6%	40-300kDa	60-200kDa	10	50µL	AP-GPG-610
						15	30µL	AP-GPG-615
			8%	25-300kDa	40-200kDa	10	50µL	AP-GPG-810
						15	30µL	AP-GPG-815
			10%	11-230kDa	20-160kDa	10	50µL	AP-GPG-1010
						15	30µL	AP-GPG-1015
			12%	6.5-200kDa	15-85kDa	10	50µL	AP-GPG-1210
						15	30µL	AP-GPG-1215
			15%	5-170kDa	10-50kDa	10	50µL	AP-GPG-1510
						15	30µL	AP-GPG-1515
			4% - 15%	5-270kDa	20-200kDa	10	50µL	AP-GPG-41510
						15	30µL	AP-GPG-41515
			4% - 20%	3.5-300kDa	5-200kDa	10	50µL	AP-GPG-42010
						15	30µL	AP-GPG-42015
Tris-Bis	MOPS or MES	1mm	8%	8-270kDa	25-245kDa	10	50µL	AP-GPB-810
						15	30µL	AP-GPB-815
			10%	8-270kDa	11-180kDa	10	50µL	AP-GPB-1010
						15	30µL	AP-GPB-1015
			12%	3.5-245kDa	11-135kDa	10	50µL	AP-GPB-1210
						15	30µL	AP-GPB-1215
			4% - 12%	3.5-270kDa	11-245kDa	10	50µL	AP-GPB-41210
						15	30µL	AP-GPB-41215

Product Name	Description	Cat. No.
Tank Adapter Plate – Type 1	Tank Adapter designed for the following models: 1. Sigma Aldrich Dual Run * 2. Thermofisher/Invitrogen XCell (I, II, & Surelock™ mini-cell) * 3. LONZA PAGEr® Minigel Chamber *	AP-AC-01
Tank Adapter Accessory – Type 2	Tank Adapter designed for the following model: 1. Life Technologies Mini Gel Tank	AP-AC-02
Ultra Fast Running Buffer for Tris-Gly Gel	Ultra Fast Running Buffer designed for Tris-Gly gel, running time as little as 20min. 1 pack for 500mL running buffer.	AP-B-P500

Instructions for Using AccuPAGE™ Precast Gel

Running Buffer Preparation

10× Stock Solution Recipe

MOPS, SDS Free 10× stock solution, 1L	MES, SDS Free 10× stock solution, 1L	Tris-Glycine, SDS Free 10× stock solution, 1L
50 mM MOPS, 50 mM Tris Base, 1 mM EDTA, pH 7.7	50 mM MES, 50 mM Tris Base, 1 mM EDTA, pH 7.3	1.92 M Glycine, 250mM Tris Base, pH8.5
1. Dissolve the following reagents in 900 mL ultrapure water. Reagent / Weight MOPS 104.6g Tris Base 60.6g EDTA 3.0g 2. Mix well and adjust the volume to 1L with ultrapure water. 3. Do NOT use acid or base to adjust the pH	1. Dissolve the following reagents in 900 mL ultrapure water. Reagent / Weight MES 97.6g Tris Base 60.6g EDTA 3.0g 2. Mix well and adjust the volume to 1L with ultrapure water. 3. Do NOT use acid or base to adjust the pH.	1. Dissolve the following reagents in 900 mL ultrapure water. Reagent / Weight Glycine 144.1g Tris Base 30.3g 2. Mix well and adjust the volume to 1L with ultrapure water. 3. Do NOT use acid or base to adjust the pH.
Add 10g SDS to the stock solution for denaturing condition.	Add 10g SDS to the stock solution for denaturing condition.	Add 10g SDS to the stock solution for denaturing condition.
Buffers are stable for 6 months when stored at 4° C.	Buffers are stable for 6 months when stored at 4° C.	Buffers are stable for 6 months when stored at 4° C.

How to Make 1× Working Solution

MOPS 1× working solution, 1L	MES 1× working solution, 1L	Tris-Glycine 1× working solution, 1L
Mix 100mL of the 10× stock solution with 900mL of ultrapure water.	Mix 100mL of the 10× stock solution with 900mL of ultrapure water.	Mix 100mL of the 10× stock solution with 900mL of ultrapure water.
Buffers are stable for 1 week when stored at 4° C.	Buffers are stable for 1 week when stored at 4° C.	Buffers are stable for 1 week when stored at 4° C.

Running Buffer Selection Guide

Gel / Buffer	Bis-Tris Denatured	Tris-Gly Denatured	Bis-Tris Native	Tris-Gly Native
Tris-Glycine	DO NOT USE.	YES Add Detergent	DO NOT USE.	YES AVOID Detergent!
MOPS	YES Add Detergent	DO NOT USE.	YES AVOID Detergent!	DO NOT USE.
MES	YES Add Detergent	DO NOT USE.	YES AVOID Detergent!	DO NOT USE.
Ultra Fast Running Buffer Cat: AP-B-P500	DO NOT USE.	YES Add Detergent	DO NOT USE.	DO NOT USE.

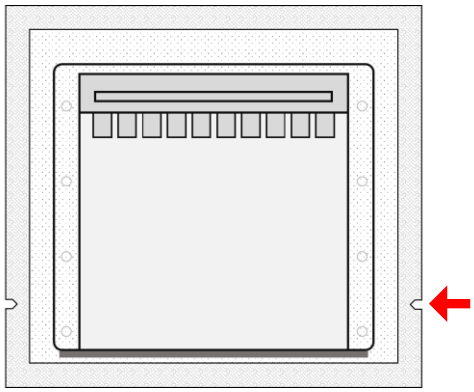
Sample Preparation

Sample and Sample Loading Buffer Volume

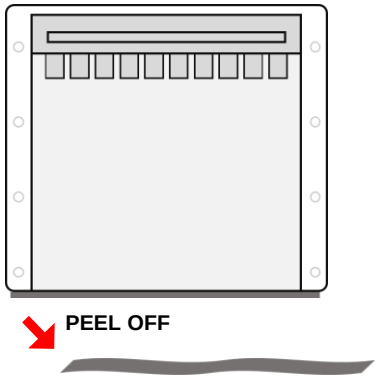
	Max Sample Volume*	2×Sample Loading Buffer	5×Sample Loading Buffer	10×Sample Loading Buffer	β-mercaptoethanol, 1M stock**	10 well precast gel final volume	15 well precast gel final volume
Denatured Sample	12μL	15μL	\	\	3μL	\	30μL
Denatured Sample	21μL	\	6μL	\	3μL	\	30μL
Denatured Sample	24μL	\	\	3μL	3μL	\	30μL
Denatured Sample	20μL	25μL	\	\	5μL	50μL	\
Denatured Sample	35μL	\	10μL	\	5μL	50μL	\
Denatured Sample	40μL	\	\	5μL	5μL	50μL	\
Native Sample	15μL	15μL	\	\	\	\	30μL
Native Sample	24μL	\	6μL	\	\	\	30μL
Native Sample	27μL	\	\	3μL	\	\	30μL
Native Sample	25μL	25μL	\	\	\	50μL	\
Native Sample	40μL	\	10μL	\	\	50μL	\
Native Sample	45μL	\	\	5μL	\	50μL	\

* The sample volume shall be adjusted according to your test. If necessary, use the same volume sample and add purified water till the indicated volume.
** Dithiothreitol (DTT) or β-mercaptoethanol (BME) can be used as a reducing agent (DTT to a final concentration of 100 mM or add BME to a final concentration of 100 mM).

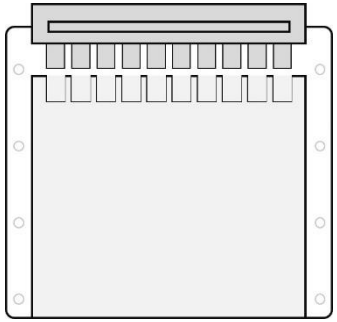
1. Open
Open the package from the indicated mark.



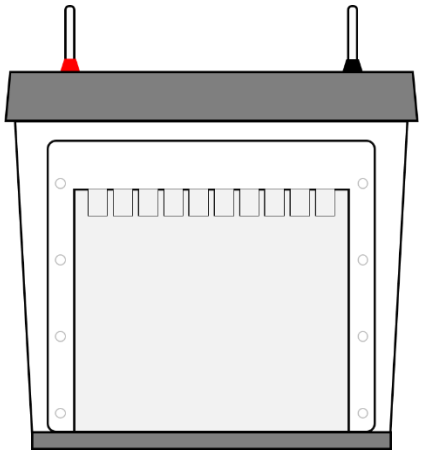
2. Peel off
Peel off the sealing tape at the bottom of the gel cassette.



3. Remove
Gently remove the comb from the gel cassette.

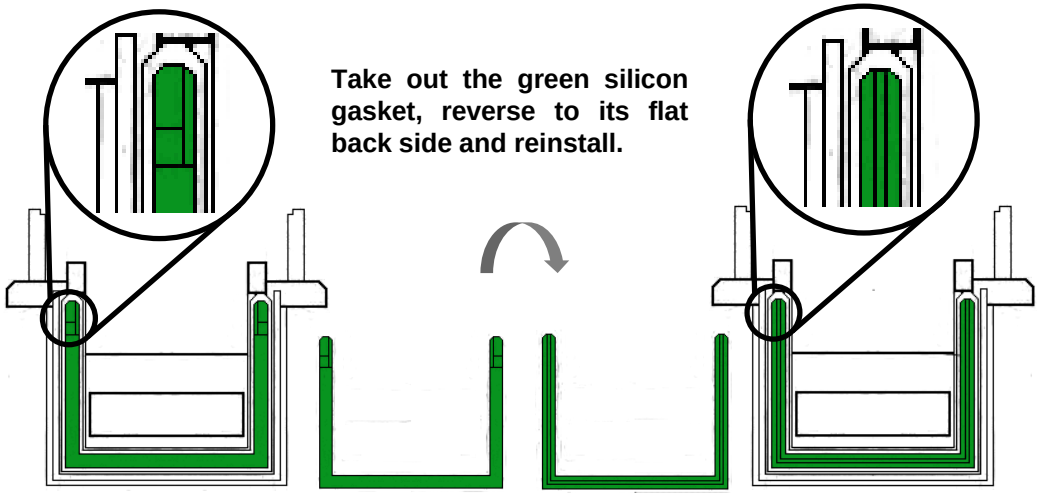


4. Gel installation
Install the gel cassette in the tank according to manufacturer's instruction.
NOTE: some brands or models of the tank need accessories for installation! Read below.



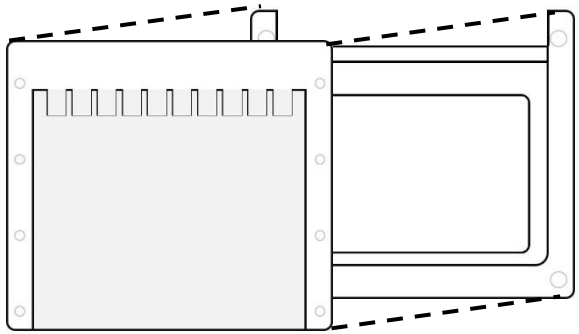
4. Gel installation – Continue, if using these brands of tank:
- **Bio-Rad Mini-PROTEAN® II and 3**
- **Bio-Rad Mini-PROTEAN® Tetra System**

Take out the green silicon gasket. Reverse to its back and put it back to the frame. Then the AccuPAGE™ Precast Gel can be well attached after installation.



4. Gel installation – Continue, if using these brands of tank:
- **Sigma-Aldrich® Dual Run and Blot System**
- **Thermo XCell I, II, & Surelock™ mini-cell**
- **LONZA PAGEr® Minigel Chamber**

These tanks are designed for thicker gel cassettes. You may use the AccuPAGE™ Tank Adapter Plate (Cat: AP-AC-01) together with the AccuPAGE™ Precast Gel to fill the gap. Use one Adapter Plate per precast gel.



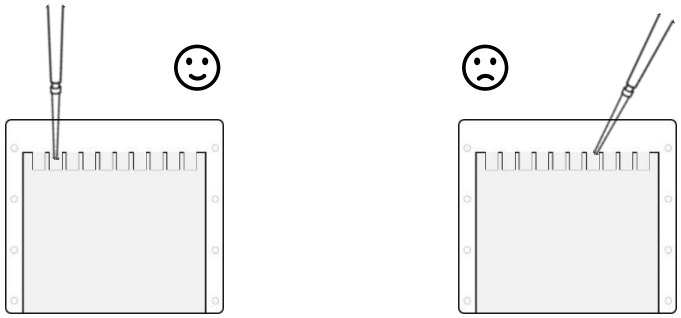
Instructions for Using AccuPAGE™ Precast Gel

5. Fill

Fill the electrode assembly with 1X running buffer to check for a proper seal prior to filling the anode (outer) chamber to the recommended level.

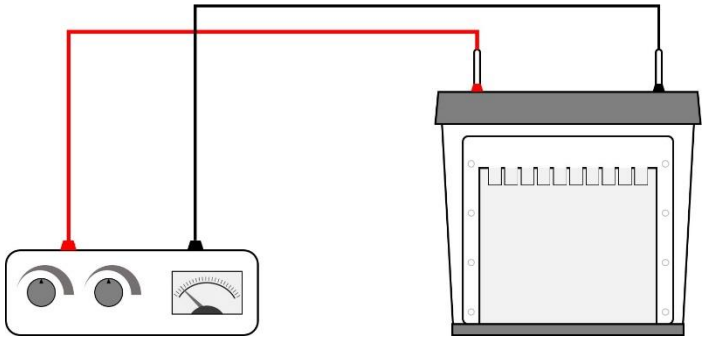
6. Load

To load the samples into the wells, insert tip vertically for better PAGE results. DO NOT EXCEED WELL CAPACITY: 50µL for 10-well gels; and 30 µL for 15-well gels.



7. Run the gel

Once the samples are loaded and the buffer is filled in the tank, place the cover over the tank and connect the cable to the power supply.



7. Run the gel - continue

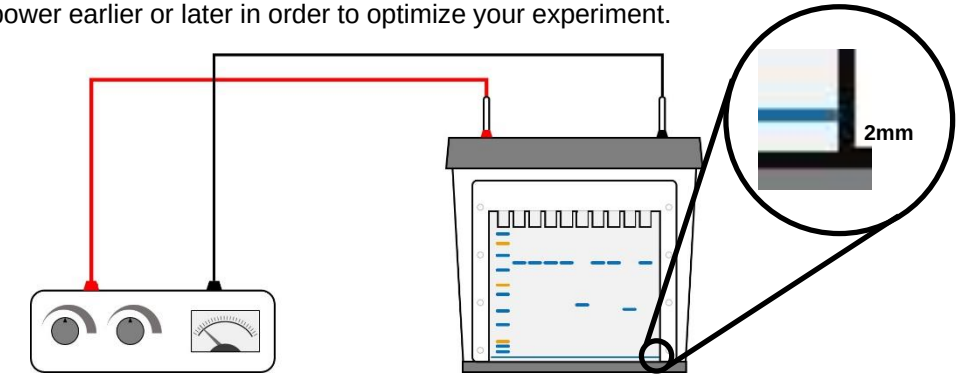
The chart hereunder shows the voltage options to run the AccuPAGE™ Precast Gel. The voltages show as ranges, you may first try with your lab typical voltage then adjust according to the chart. During the running process, the voltage shall not be changed.

It is ideal to run PAGE at the temperature range of 10°C - 20°C. However, during the run, the electric current will increase and more heat will be generated. If the temperature of the running buffer goes above 30°C, please put the tank in ice to keep cool.

	Running Buffer	Typical Voltage	Typical Run time	Optional Voltage	Optional Run time
Bis-Tris Gel **General condition**	MOPS	150V-200V	25-50min	\	\
Bis-Tris Gel **To obtain more clear and sharp bands**	MOPS	\	\	100V-150V	45-60min
Bis-Tris Gel **General condition**	MES	150V-180V	25-45min	\	\
Bis-Tris Gel **To obtain more clear and sharp bands**	MES	\	\	100V-150V	40-60min
Tris-Glycine Gel **General Condition**	Tris-Gly	160-180V	50-60min	\	\
Tris-Glycine Gel **To obtain more clear and sharp bands**	Tris-Gly	\	\	120V-150V	60-90min
Tris-Glycine Gel **General Condition**	Ultra Fast Running Buffer AP-B-P500	250V	18-25min	\	\

8. Stop

Stop the power when the blue staining line is 2mm from the edge. You may stop the power earlier or later in order to optimize your experiment.



Instructions for Using AccuPAGE™ Precast Gel

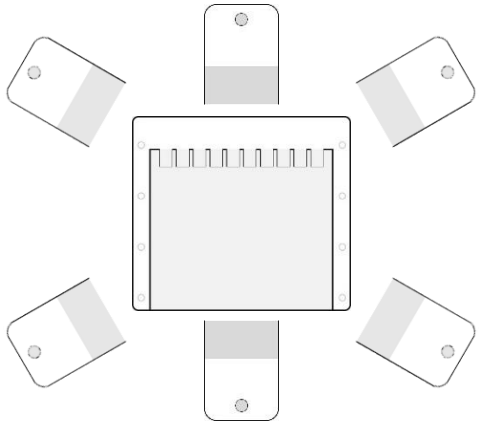
9. Open

Open the cassette to take out the gel.

Once the electrophoresis is finished, you may take out the gel cassette from the electrode assembly. Use the Gel Cassette Opener to open the gel cassette, from the corners as well as top and bottom. OPERATE WITH CAUTION!

After the cassette cover is opened, use the Gel Opener to cut softly the left and right side of the gel to remove the gel.

If necessary, put the gel cassette in water flow or water bath to help you open the cassette and remove the gel.



10. Staining

AccuPAGE™ Precast Gel is compatible with common staining protocols. You may select either lab made staining or commercial staining. Please operate the staining by following your lab protocol or the commercial instructions.

11. Western Blot.

AccuPAGE™ Precast Gel is designed to adapt with popular WB protocols, including both wet transfer and semi-dry transfer. In order to achieve good initial test result, please follow the blotting equipment manufacturer's instruction.

Notes to help you achieve better result:

1. Always use pre-stained marker during transfer. It will let you see the transfer result directly.
2. Both PVDF and NC membranes are compatible with AccuPAGE™ Precast Gel.
3. Do NOT reuse the transfer buffer while following wet transfer protocol.
4. Avoid short circuit of the power supply. Make sure that: membrane is smaller than the gel, filter paper is smaller than the membrane, foam (wet transfer) is smaller than the filter paper.

Troubleshooting Guide

Problem	Probable Cause	Solution
Protein bands distorted.	Air bubble in the sample well.	1. When filling the running buffer, flush the wells with the pipette. 2. Before loading the sample, centrifuge the sample to remove the air bubble. 3. During sample loading, avoid air entering.
	Buffer enters the gel cassette because of broken.	Change to a new cassette.
Tracking dye part or in whole switch colour to yellow.	pH value decreased.	Prepare fresh running buffer according to recipe. Check the pH before running.
Streaking of the protein bands.	Protein aggregates, Insoluble or weakly charged particles (such as carbohydrates) exist in the sample.	1. Dilute the protein sample to avoid aggregates. If problem continues, try other protein solutions. 2. Heat sample in the existance of SDS then centrifuge and take only the supernatant for testing.
	The sample contains high concentration of salt.	Dialysis or ultrafiltration to remove the salt.
	The protein sample contaminated by DNA complex or membranes.	Centrifuge the protein sample and take only the clear supernatant.
Electrophoresis time is unreasonable long.	The sealing is not removed from the bottom of the cassette.	Remove the sealing tape before installing the cassette.
	Running buffer in the electrode assembly is connected to the buffer in the tank.	Check the electrode assembly for leaking. Reinstall it if necessary.
	1. Running buffer was made wrong.	1. Remake the running buffer according to the recipe. 2. If using the buffer concentrate, please dilute it correctly.
	Incorrect running conditions.	1. Use constant voltage and do not limit the electric current. 2. Use a power supply that is generating sufficient electric current.
Bands are not well separated.	Incorrect gel percentage.	Choose the proper gel concentration according to the protein separation chart.
	Incorrect running buffer.	Select the running buffer according to the gel type. (Chart: running buffer selection guide.)
	Sample overloaded.	Reduce the concentration of the protein sample.
	Incorrect sample buffer.	Use freshly made sample buffer and operate according to the instruction.
	PAGE system is over heat.	1. Check the voltage and the electrical current, verify the setting is correct. 2. Cool down the tank by ice bath, adding ice pack. 3. Please refer to the manufacturer's instruction.
The preset voltage can not be reached; or the electrical current is over high.	Running buffer in the electrode assembly is connected to the buffer in the tank.	Check the electrode assembly for leaking. Reinstall it if necessary.
	The electrophoresis tank was assembled wrong.	Check the manual of the tank and reassemble the tank.
	The sample contains high concentration of salt.	Dialysis or ultrafiltration to remove the salt.

To be continued.

Troubleshooting guide - continue

Problem	Probable Cause	Solution
Low or no electrical current during the electrophoresis.	The electrical circuit is not well connected.	1. Check if the tape under the cassette has been removed, if not please removed it. 2. Make sure the running buffer covers the sample wells. 3. Check all of the cable connections and make sure they are well connected: electrode assembly, cable and the power supply.
Air bubble appears between the gel and the cassette.	PAGE system is over heat.	1. Check the voltage and the electrical current, verify the setting is correct. 2. Cool down the tank by ice bath, adding ice pack. 3. Please refer to the manufacturer's instruction
Protein bands show like the shape of dumbbell after electrophoresis.	The sample volume is too large and affected the sample stacking.	Load proper volume of sample in each well.

Notice

All of the information and advices provided in the user guide are based on the best of our knowledge and ability, but without obligation or liability. Our information and advice do not exempt our customers own responsibility for checking the suitability of the products for intended purpose.
The information in the user guide is subjected to change from time to time, without prior notice and should not be entitled as a commitment by manufacturing or selling entity, or an affiliate. We deny any responsibility for any errors that may appear in this document.

Contact information

Please contact the sales agency from where you have purchased the product.
Or you may send email to info@bio-bench.com for any question.

Standard Warranty

12 months warranty from the date of production.