p-PAGEL Instruction Manual

Sep 8th,2022 Ver.4

1. Safety warnings and precautions

To use this product safely, please read this instruction manual carefully first. The complete instructions should be read and fully understood before use of the product. The procedure described in the instruction manual applies only to the use for the intended purpose. Using the product for any purpose other than the intended use or in any manner other than that described in the manual are prohibited. User shall be liable for all safety measures needed for any use other than specified in the manual. Also, please read carefully and understand the instruction manuals for the products to be used at the same time.

2. Introduction

"p-Pagel" is pre-cast polyacrylamide gel (Tricine gel system) for low molecular weitght-protein and peptide electrophoresis. The tricine gel system electrode buffer and the electrophoresis system (for pre-cast gels) dedicated for ATTO mini-size gels are required to use.

3. Package

Name	Size		Qty
p-PAGEL	Gel size	90(W)x83(H)x1mm(t)	10
	Glass plate size	120(W)x100(H)x2mm(t) (Total 5mm)	plates/ box

4. Components

Name	Main component
p-PAGEL	Polyacrylamide gel

This product doesn't contain any notifiable material exceeding to regulated amount for exclusion decided by PRTR Law, Poisonous and Deleterious Substances Control Act, and Industrial Safety and Health Law.

5. Preservation method

- Keep refrigerated. Freezing will damage the quality of the product.
- The expiration date is indicated on the outer box and on the gel package.

6. Disposal method

- When disposing of reagents and plates, follow the disposal rules of your institution.

Material Plate: Glass / Packaging: PET Nylon

7. Necessary things other than this product

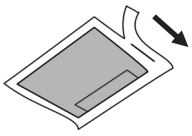
- ATTO electrophoresis apparatus for mini size gels
- Power supply（175 V, 160 mA or more output recommended）
- Tris/Tricine/SDS Electrode buffer etc.

8. Precautions for use

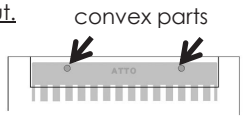
- Refrigeration at 5-10℃ is recommended for storage. Never place this product near a cold air outlet, as it may freeze even in a refrigerator.
- Please note that if frozen, the product will not be usable because of deformation due to air bubbles, plate peeling, swelling and shrinkage, etc.
- Please open the package just before use. The quality will deteriorate after opening, so please use it immediately.
- Handling this product with bare hands may cause injury. Wear rubber gloves and protective clothing when handling.

9. Usage

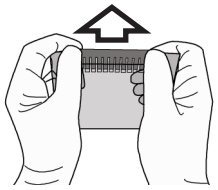
9-1. Preparation of gels and electrode buffer



1. Open the package and take out the gel.
   ＊ If it's difficult to cut, use scissors.
   ＊ Please note that the gel may peel off from the glass plate if the gel is forcibly pulled out.



2. Slowly pull out the comb.
   ＊ Put your fingers on the two convex parts on the surface of the comb, align the left and right sides little by little, and slowly remove the comb. Be careful not to bend or cut the wells.



3. Prepare the electrode buffer EzRun T (Tris/ Tricine/SDS).
4. Wash the wells with electrode buffer.

9-2. Electrophoresis

1. Set the gel in the electrophoresis unit dedicated to ATTO mini-size gel, and add the electrode buffer solution.
   ＊ Set the gel according to the instruction manual attached to the electrophoresis apparatus.
2. Apply appropriate volume of sample to each well.
   * The maximum apply volume is described as about 60% of the maximum capacity of the wells.

Code	Model	Comb (wells)	Well size	Maximum apply volume
232260	P-T16.5S	14 wells	4.2(W)×10(H) mm	24μL
232265	P-R16.5S	18 wells	2.9(W)×10(H) mm	18μL

3. Set the power supply with reference to the table below.

	Voltage	Current	Time
C.V.	**175 V setting**	At start：70-90 mA At end：30-40 mA	60-75 min.
	150 V setting	At start：55-75 mA At end：25-35 mA	90-110 min.
C.C.	At start：70-85 V At end：180-200 V	**40 mA/gel setting**	80-100 min.
	At start：150-165 V At end：230-250 V	**60 mA/gel setting**	55-70 min.

＊ C.C. ; Constant Current / C.V. ; Constant Voltage

*When using constant current (constant voltage) setting, set the voltage value (current value) referring to the table above.

*Please note that the buffer solution will be hot after electrophoresis.

*Time, current and voltage values at start and end (actual measured values) shown in the table are for your reference only. It varies depending on gel concentration and other factors.

*For constant voltage setting, set the voltage to 175V or 150V regardless of the quantity of gels.

*For constant current setting, calculate the quantity of gels ×60 mA/40 mA and double the current value.

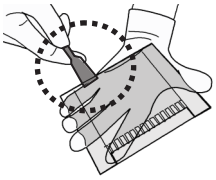
*When using PageRun Ace, set "Std GEL2" (42 mA c.c.) for approximately 60 minutes for one gel and "HiGEL2" (24 w c.w.) for approximately 90 minutes for two gels. If you set "HiGEL1" for one gel,

the electrophoresis will be completed in about 40 minutes, but the pattern may be affected due to the high temperature of the buffer.

4. Turn on the power supply and start electrophoresis.

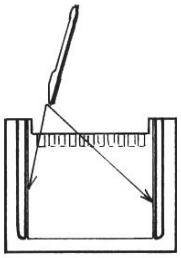
9-3. End of electrophoresis

1. When the dye front (CBB) reaches about 5 to 10 mm from the bottom edge of the gel, turn off the power supply and stop running.



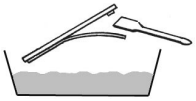
∗ Please complete the electrophoresis operation according to the instruction manual attached to the electrophoresis device.

2. Remove the gel plate from the electrophoresis chamber, insert a flat tool such as a spatula between the glasses and gently move it up and down to open the gel plate. Remove one piece of glass plate on the top.



3. Use a spatula or scalpel to make an incision between the gel and the side of the spacers.

∗ If the spatula is wet, it will move smoothly and prevent damage to the gel.

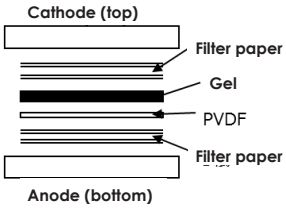


4. Transfer the gel to a container filled with staining or fixing solution. Hold the gel plate with the gel side facing down, insert a spatula between the gel and gel plate, and peel off the gel.
5. Gently shake the container to soak the entire gel in the staining solution.

∗CBB staining, silver staining, reverse staining, fluorescent staining, etc. can be used.

∗For clear pattern detection of low molecular weight proteins, staining with EzStainAQua is recommended. Use the microwave method to prevent loss of low molecular weight proteins. If hand-cast CBB stain is used, soak the samples in 50% methanol/10% acetic acid fixing solution for 20-30 minutes, then stain with CBB.

9-4. Western blotting



1. The PVDF membrane is hydrophilized with methanol and equilibrated with blotting buffer. The filter papers are also soaked in blotting buffer.
2. Wash the gel after electrophoresis with blotting buffer.
3. Referring to the figure on the right, set the filter papers, PVDF membrane, and gel on top of each other in the blotting apparatus.
4. Remove excess solution and air with a roller.
5. Refer to the table as follows to start energizing and transferring.

		Filter paper	Voltage	Current	Time
Standard	c. c.	2-3 sheets × top and bottom	40 V	0.144 A/gel	EzBlot, EzFastBlot, HMW :30-60 min.
	c. v.		12 V	0.5 A/gel	
High speed	c. c.		40 V	0.45 A/gel	EzFastBlot: 10-15 min.
	c. v.		20V	0.5 A/gel	HMW: 15-30 min.
QBlot kit M	c.v.	Unnecessary	12V	0.1~0.4A	15-30 min.
			24V	0.5~0.8A	5-10 min.

∗For transferring low-molecular-weight proteins, use PVDF membranes with small pore size (Clear Blot P/Plus membrane 0.2 µm).

∗Adding 5–20% methanol to the blotting buffer prevents loosing of low molecular weight molecules from the PVDF membrane during transfer.

∗Air-drying the PVDF membrane after transfer may reduce the loss of low molecular weight proteins from the membrane. Air-dried PVDF membranes can be stored at -20°C between filter papers or sealed in zip lock bags. When using the membranes, soak them in 100% methanol for a few seconds to hydrophilize them, wash them with TBS-T, and start antibody reaction from blocking treatment.

Please note that if the PVDF membrane is left in the solution for a long time after transfer, low-molecular-weight proteins may be lost.

10. Reference

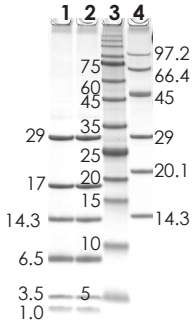
How to prepare samples for low molecule electrophoresis

The following is a brief description of the method according to the Shagger method. Dissolve the sample in the following sample buffer and heat at 37°C for 15 to 60 minutes.

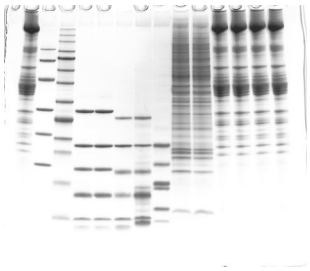
4×SDS sample buffer ：12% SDS (w/v), 6% mercaptoethanol (v/v), 30% glycerol (w/v), 0.05% Coomassie blue G-250, 150 mM Tris/HCl (pH 7.0)

Electrophoresis pattern

ATTO Molecular Weight Marker



Lane 1,2：*EzStandard LMW*
Lane 3：*EzProtein Ladder*
Lane 4：*EzStandard*



Lane 1,11-14：Chicken muscle extract
Lane 2：*EzStandard*
Lane 3：*EzProtein Ladder*
Lane 4,5：*EzStandard LMW*
Lane 6-8：Other company peptide markers
Lane 9,10：Hela extract

Electrode buffer：*EzRun T*
Electrophoresis conditions：Running gel at 175V c.v. for about 60 min.
Gel staining: Gels were immersed in 50 mL of EzStain AQUA, microwaved (600 W for 45 seconds), and stained while shaking for about 2 hours. Then, it was replaced with water and destained in a microwave oven (600 W, 30 seconds) until the gel became transparent.

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