p-PAGEL Instruction Manual

July 26th, 2018 Ver. 2

1. Safety precautions

Before using the product, read this manual thoroughly at first. Do not start the operation until you understand the contents of manual. This document explains only methods utilized for specified purposes. Do not use the product for any purpose or by any method not described in this manual. If it is used for any purpose or by any method not described in this manual, an operator should take responsibility for all required safety measures and contingencies. Also, read a manual of equipment used with it simultaneously.

2. Application purpose

「p-PAGEL」is a precast polyacrylamide gel (Tricine type) for electrophoresis of low protein and peptide. A tricine-based running buffer and an electrophoresis device (precast gel specification) exclusively for ATTO mini-gel size are required.

3. Package

Product name	Size	Package
p-PAGEL	Gel size 90(W)x83(H)x1mm(t) Glass plate size 120(W)x100(H)x2mm(t)(total 5mm)	10/pk

4. Components

Product name	Major components
p-PAGEL	Polyacrylamide gel

This product doesn't include a 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

- Please keep refrigerated. Quality is impaired when freezing.
- The expiration date is displayed on the outer box and gel packaging bag.

6. Disposal method

- Follow a disposal method decided by the organization you belong to.

  Materials Plate : Glass Packaging : PET nylon

7. Necessary things other than the product

- Electrophoresis apparatus for ATTO Mini-gel size
- Power supply (recommended output over 175 V, 160 mA)
- Tris/Tricine/SDS electrophoresis buffer etc.

8. Precautions for use

- Please keep refrigerated. (5~10℃) Please do not place near the cold air outlet. It may freeze.
- Freezing causes deformation such as bubbles, plate peeling, swelling and shrinkage, and can not be used.
- Please open the bag just before use. After opening, please use it immediately as the quality deteriorates.
- Handling the gel with bare hands may cause injury. Please wear gloves and protective clothing when handling.

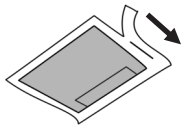
9. Usage

9-1. Preparation of gel and electrophoresis buffer

1. Open the packaging bag and take out the gel.

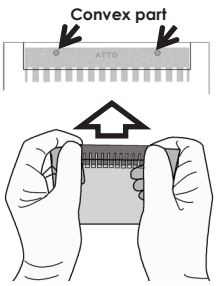
   ※When it is difficult to cut, please cut it with scissors.

   ※If you pull out the gel forcibly, the glass will peel off from the gel. Please avoid it.



2. Pull out the comb slowly.

   ※Place your fingers on the two convex parts of the comb and slowly remove the comb by aligning the left and right sides. Remove the comb without bending or cutting the wells.



3. Prepare the electrophoresis buffer ***EzRunT*** (Tris/Tricine/SDS).
4. Wash wells with electrophoresis buffer.

9-2. Electrophoresis

1. Place the gel in an electrophoresis device exclusively for ATTO mini-gel size and pour the electrophoresis buffer.

   ※Please set the gel according to the instruction manual attached to the electrophoresis device.

2. Apply the appropriate amount of sample to each well.

   ※The maximum applied amount is expressed as about 60% of the maximum capacity of the well.

Code	Type	Comb (Number of wells)	Well size	Maximum applied amount
232260	P-T16.5S	14 wells	4.2(W)×10(H) mm	24 µL
232265	P-R16.5S	18 wells	2.9(W)×10(H) mm	18 µL

3. Set the power supply referring to the following table.

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

※C.C.; Constant Current, C.V.; Constant Voltage

※In case of C.V. / C.C., set the allowable voltage / current value refer to the table.

※After electrophoresis, buffer solution can be hot, so please be careful.

※The current or voltage value (measured value) other than the setting values are the standard. It depends on the gel concentration.

※For constant voltage setting, please set it to **175 V/150 V** regardless of the number of gels.

※For constant current setting, calculate "the number of gels x **60 mA/40 mA**" and double the current value.

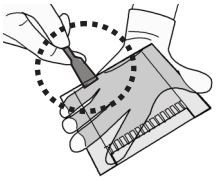
※In the case of using **PageRun Ace**, run one gel on『**Std GEL2**』(42 mA c.c.) for about 90 min, run two gels on『**HiGEL2**』(24 W C.W.) for

about 60 min. If you run one gel on 『**HiGEL1**』, the electrophoresis will be completed in about 40 min, but the pattern may be affected because the buffer becomes high temperature.

4. Power is applied to perform electrophoresis.

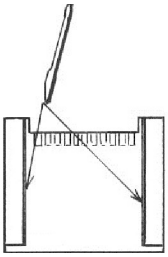
9-3. End of electrophoresis

1. Stop migration when color marker (BPB) reaches to the 5~10 mm upper position from the lower end of the gel.



※Please follow the instruction manual attached to the electrophoresis device.

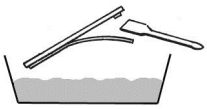
2. Remove the gel plate from the electrophoresis tank, insert flat objects such as a spatula between the glass, move it gently up and down to open the gel plate. Remove the top glass plate.



3. Make a cut between the gel and the spacer side with a spatula or scalpel.

※If you moisten the spatula, it will move smoothly, and you can prevent damage to the gel.

4. Transfer the gel to the vat containing the staining or fixing solution. Hold the gel plate with the gel side facing down, insert a spatula between the gel and the plate, and peel off the gel.



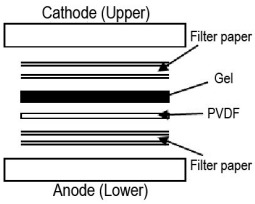
5. Immerse the whole gel in the staining solution.

※CBB/silver/reverse/fluorescent staining, etc. can be use.

※***EzStainAQua*** is recommended for sensitive detection of low molecular weight (LMW) proteins. Use microwave to prevent elution of LMW protein. If you using a hand-made CBB staining, soak in the 50% methanol or 10% acetic acid for 20-30 min before dyeing the CBB to fix the proteins.

9-4. Western blotting

1. The PVDF membrane is hydrophilized with methanol and equilibrate with blotting buffer. Immerse the filter paper in the blotting buffer.
2. Wash the gel after electrophoresis with a blotting buffer.
3. Place the filter paper, gel, and PVDF membrane on the blotting device refer to the right figure.



4. Remove excess buffer and bubbles with the roller.
5. Start the transfer refer to the table below.

		Filter paper	Voltage	Current	Time
Standard	c. c.	2~3 sheets each of cathode and anode	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	c.v.	Not required	12V	0.3~0.8A	15~30 min
			24V	1~1.6A	5~10 min

※Please use PVDF membrane with a small pore size (0.2 um) for transfer of LMW protein.

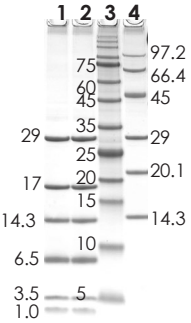
※When 5~20% methanol is added to the blotting buffer, LMW proteins will not easily fall off from the PVDF membrane during transfer.

※If the PVDF membrane is dried after the transfer, it may reduce loss of LMW protein from the membrane. Put the dried PVDF membrane into filter paper, seal it in a zipped bag and store it at –20℃. For use, please immerse in 100% methanol for several seconds to hydrophilized, wash with TBS-T and start antibody reaction from blocking treatment. Also, please note that LMW proteins may fall off if the PVDF membrane is left in the solution for a long time.

10. Reference

Electrophoresis pattern

ATTO molecular weight marker



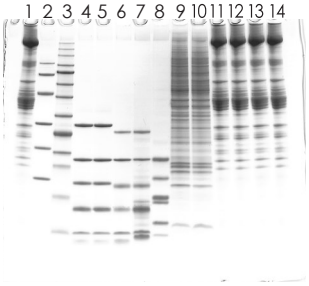
Lane 1,2：***EzStandard LMW***

Lane 3：***EzProtein Ladder***

Lane 4：***EzStandard***

Electrophoresis buffer: ***EzRun T***

Example of electrophoresis stained with *EzStainAQua*



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

Electrophoresis buffer: ***EzRun T***
Conditions: 175 V C.V. 60 min
Gel staining: Immersed in 50 mL of ***EzStain AQua***, stained with microwave (600 W, 45 sec) and shaking for about 2 hr. After that it was replaced with water and destained by microwave (600 W, 30 sec)

11. Contact us

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