u-PAGEL H Instruction Manual

June 28th, 2021 Ver.1

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

'u-PAGEL H' is pre-cast polyacrylamide gel (Tris-Gly system) for electrophoresis of high molecular weight proteins and nucleic acids. Please use our electrophoresis system (for pre-cast gels) dedicated for mini-size gels.

3. Package

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

Model	Gel concentration	Molecular weight separation range	
		Protein (kDa)	Nucleic acid(bp)
UH-T/R5	5%	75 -1,000	500 - 5,000
UH-T/R310	3 -10%	35 -1,500	200 - 5,000
UH-T/R420	4 - 20%	5 - 600	30 - 25,00

Electrode buffer : **Protein**： ***EzRun*** (Tris/Glycine/SDS)
Nucleic acid： ***EzRun TG*** (Tris/Glycine)

4.Components

Name	Main component
u-PAGEL H	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

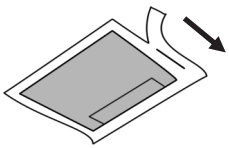
- ATTO electrophoresis apparatus for mini size gels
- Power supply（300V, 150mA or more output recommended）
- Electrode buffer etc.

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.
- The glass plate of this product cannot be reused. Dispose of it in accordance with the disposal rules of your institution.

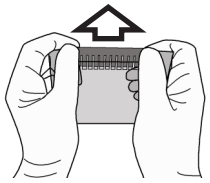
9. Usage

9-1. Preparation of gels and electrode buffer



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

2. Slowly pull out the comb.
   ∗ Put your finger s 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 corresponding to the sample treatment (with or without SDS treatment) and gel type.

   - **SDS-PAGE**
     EzRun (Tris/Gly/SDS) or ***EzRun MOPS*** (Tris/MOPS/SDS)*
     ∗EzRun MOPS is not recommended for 5% and 3-10% gels because it reduces the separation range of high molecular weight proteins.
   - **Native PAGE / DNA electrophoresis**
     EzRun TG (Tris/Gly)

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 an appropriate volume of sample to each well.
   ∗ The maximum apply volume is described as about 60% of the maximum capacity of the wells.

Comb (wells)	Well size	Maximum apply volume
14 wells	4.2(W)×10(H) mm	24μL
18 wells	2.9(W)×10(H) mm	18μL

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

4-20%

Electrode buffer		Voltage	Current	Time
EzRun Tris/Glycine/SDS	C.V.	**300V setting**	At start : 60-75mA At end : 30-40mA	30-40 min
	C.V.	**150V setting**	At start : 25-35mA At end : 10-20mA	70-80 min
	C.C.	At start : 90-110V At end : 190-210V	**20mA/gel setting**	75-85 min
EzRun MOPS Tris/MOPS/SDS	C.V.	**250V setting**	At start : 60-80mA At end : 30-40mA	25-35 min
	C.V.	**150V setting**	At start : 30-40mA At end : 10-20mA	55-65 min
	C.C.	At start : 70-85V At end : 150-160V	**20mA/gel setting**	75-85 min
EzRun TG (Nucleic acid) Tris/Glycine	C.C.	At start : 100-120V At end : 250-270V	**20mA/gel setting**	65-85 min

3-10%

Electrode buffer		Voltage	Current	Time
EzRun Tris/Glycine/SDS	C.V.	**300V setting**	At start : 90-110mA At end : 40-50mA	35-45 min
	C.V.	**150V setting**	At start : 30-50mA At end : 15-25mA	90-100 min
	C.C.	At start : 50-70V At end : 110-130V	**20mA/gel setting**	100-120 min
EzRun TG (Nucleic acid) Tris/Glycine	C.C.	At start : 70-90V At end : 180-200V	**20mA/gel setting**	90-110 min

5%

Electrode buffer		Voltage	Current	Time
EzRun Tris/Glycine/SDS	C.V.	**300V setting**	At start : 90-100mA At end : 40-50mA	35-45 min
	C.V.	**150V setting**	At start : 40-50mA At end : 10-20mA	90-110 min
	C.C.	At start : 50-70V At end : 150-170V	**20mA/gel setting**	100-120 min
EzRun TG (Nucleic acid) Tris/Glycine	C.C.	At start : 70-90V At end : 180-200V	**20mA/gel setting**	90-100 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.

*Time, current and voltage values at start and end (actual measured values) are for your reference only.

*For constant voltage setting, set the voltage to 150V or 300V regardless of the quantity of gels. For constant current setting, calculate the quantity of gels x 20mA and set the current value. For example, if there are 2 gels, set the value to 40 mA.

*For high-speed electrophoresis of high-molecular weight proteins of 200 kDa or more, run at 100 V constant voltage for 5 to 10 minutes, and after the sample is in the gel, run electrophoresis at 300 V constant voltage setting.

*When using PageRun Ace (WSE-1150), please refer to the table below for setting the conditions.

Electrophoresis condition *EzRun* Tris/Glycine/SDS	Time		
	4-20%	**3-10%**	**5%**
Hi GEL1 (24W c.w.)	20-30 min	30-40 min	30-40 min
Hi GEL2 (24W c.w.)	30-40 min	40-50 min	40-50 min
Std GEL1 (21mA c.c.)	60-70 min	95-105 min	95-105 min
Std GEL2 (21mA c.c.)	65-75 min	100-110 min	100-110 min

4. Turn on the power supply and start electrophoresis.

※Please note that the temperature of the electrode buffer may become high during energization.

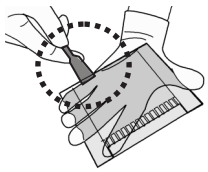
Depending on the number of gels, the buffer temperature may rise to around 30°C in the case of 150 V constant voltage and 20 mA/gel constant current setting, and to around 50°C in the case of 300 V constant voltage setting or HiGEL 1 or 2 setting with PageRunAce (WSE-1150).

9-3. End of electrophoresis

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

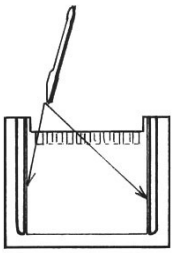
* Please complete the electrophoresis operation according to the instruction manual attached to the electrophoresis device.

2. Remove the gel plate from the electrophoresis chamber and remove the gel from the gel plate. 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 on the top.

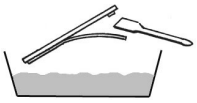


3. Make a notch between the gel and the spacer with a spatula or scalpel moistened with electrode buffer or staining solution.

* If the spatula is dry, the gel will easily stick to the spatula and the gel may be damaged.



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 the gel plate, and peel off the gel.



5. Gently shake the container to soak the entire gel in the staining solution. If the container is shaken vigorously, the gel may fold and stain unevenness may occur.

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

9-4. Western blotting

1. The PVDF membrane is hydrophilized with methanol and equilibrated with transfer buffer.
2. Cut off the wells of the gel after electrophoresis and wash with transfer buffer.
3. Place 2-3 sheets of filter paper (2-3 mm thick) soaked in transfer buffer on the anode side of the electrode plate.

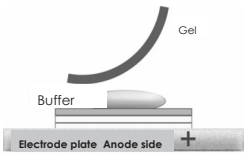
4. Place a PVDF membrane on the top of filter papers and press with a roller.

5. Drop off 5 -10 mL of transfer buffer to the central part of the PVDF membrane.
6. Refer to the following steps a to c, place the gel on top of the transfer buffer so that it floats and fits into the four corners of the membrane.

   *The top edge of the gel may fold due to low gel concentration.

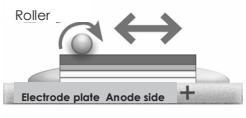
   a. Hold the bottom edge of the gel and place the top edge of the gel on the transfer buffer.

   b. Leave the top of the gel floating above the transfer buffer and align the wells of the gel with the edges of the PVDF membrane.

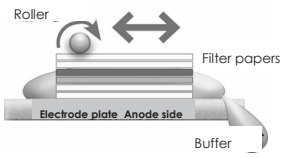


   c. After placing the top edge of the gel on the PVDF membrane, lay the gel on top of it so that it fits the other four corners of the PVDF membrane.

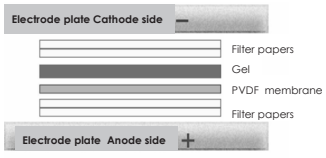
7. Remove excess solution by pressing down lightly with a roller. If too much pressure is applied at this time, the gel may deform.



8. Place 2-3 more filter papers on top of the gel.
9. Remove excess solution and air with a roller. If too much pressure is applied at this time, the gel may deform.



10. Place the electrode plate on the cathode side, set the blotting device, and connect it to the power supply.



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

*The voltage and current settings and transfer time when using the following transfer buffers are listed below.

AE-1460 EzBlot , AE-1465 EzFastBlot, WSE-7210 EzFastBlot HMW

WSE-4057 QBlot kit M

* The current (voltage) value and time for the set voltage (current) value are for reference only.
* EzBlot or EzfastBlot HMW is recommended for transcription of high molecular weight proteins.
* For UH-310, constant current or 12V constant voltage setting is recommended. Transfer under high-speed blotting setting may cause the top edge of the gel to adhere to the PVDF membrane.

4-20%

		Filter paper	Voltage	Current	Time
Standard	c. c.	2-3 pcs × Top and bottom	5-25 V	0.144 A/gel setting	30-60min
	c. v.		12V setting	0.1-0.5A/gel	
High - speed	c. c.		10-30V	0.45 A/gel setting	EzFastBlot 10-15min HMW 15- 30min
	c. v.		20-25V setting	0.2–1.0A/gel	
QBlot kit M	c.v.	Not required	12V setting	0.2-0.6A	15- 30min
			24V setting	0.6-1.0A	5-10min

3-10%

		Filter paper	Voltage	Current	Time
Standard	c. c.	2-3 pcs × Top and bottom	5-20 V	0.144 A/gel setting	30-60min
	c. v.		12V setting	0.1-0.6A/gel	
QBlot kit M	c.v.	Not required	12V setting	0.2-0.8A	15- 30min

5%

		Filter paper	Voltage	Current	Time
Standard	c. c.	2-3 pcs × Top and bottom	5-30 V	0.144 A/gel setting	30-60min
	c. v.		12V setting	0.1-0.9A/gel	
High - speed	c. c.		10-30V	0.45 A/gel setting	EzFastBlot 10-15min HMW 15- 30min
	c. v.		20-25V setting	0.1–1.3A/gel	
QBlot kit M	c.v.	Not required	12V setting	0.2-0.6A	15- 30min
			24V setting	0.6-1.3A	5-10min

12. Start energizing and transferring.

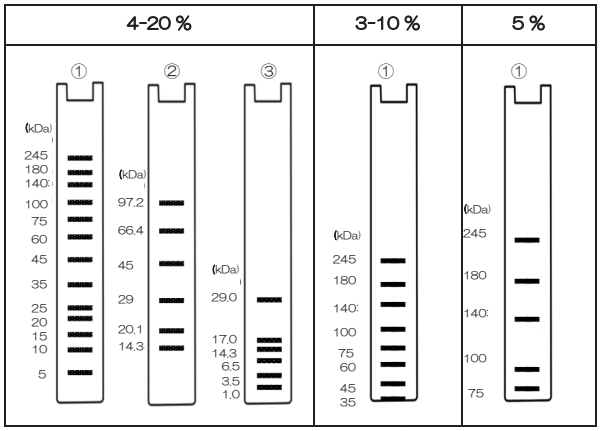
* The PVDF membrane after transcription is used for membrane staining, from blocking to antibody reaction.

10. Reference

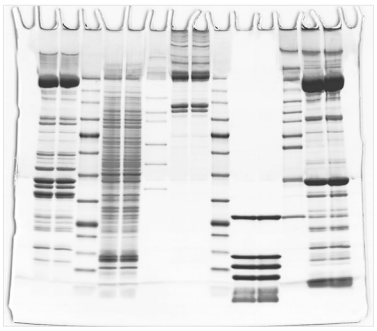
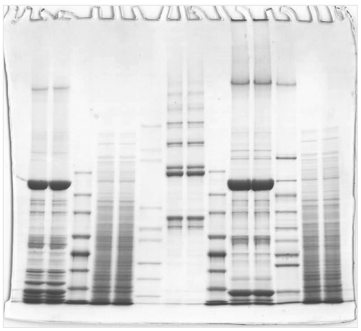
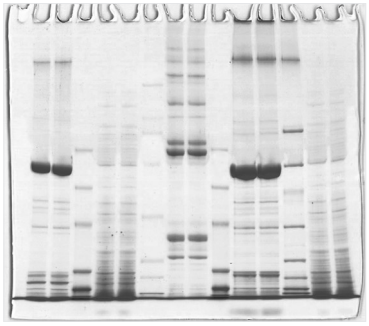
10-1. Electrophoresis Pattern　Schematic diagram showing the mobility of the band

EzRun (Tris/Glycine/SDS)
Using a electrode buffer

① ***EzProteinLadder***
② ***EzStandard***
③ ***EzStandard LMW***



10-2. Electrophoresis pattern　Example of electrophoresis stained with EzStainAQua (CBB staining solution)

4-20 %	3-10 %	5 %
		
Lane 1,2,13,14 : Chicken muscle extract Lane 4,5 : Hela extract Lane 7,8 : Collagen extract Lane 10,11 : ***EzStandard LMW*** Lane 6 : Molecular weight marker Lane 12 : Molecular weight marker Lane 3,9 : ***EzProteinLadder***	Lane 1,2,10,11 : Chicken muscle extract Lane 4,5,13,14 : Hela extract Lane 6 : Molecular weight marker Lane 12 : Molecular weight marker Lane 7,8 : Collagen extract Lane 3,9 : ***EzProteinLadder***	Lane 1,2,10,11 : Chicken muscle extract Lane 4,5,13,14 : Hela extract Lane 6 : Molecular weight marker Lane 12 : Molecular weight marker Lane 7,8 : Collagen extract Lane 3,9 : ***EzProteinLadder***

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