Pre-made polyacrylamide gel e-PAGEL HR

e-PAGEL HR Instruction Manual

Oct 20th, 2016 Ver.3

1. Safety precautions

Before using the product, read this manual thoroughly at first. Do not start the operation until you understand the contents of this manual. This manual 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

[e-PAGEL HR] is a pre-made polyacrylamide gel for electrophoresis of protein and nucleic acid. An electrophoresis device exclusively for ATTO Mini-gel size are required.

3. Package

Product name	Size	Package
e-PAGEL HR	Gel size 90 (W) x 83 (H) x 1 mm (t) Glass plate size 120 (W) x 100 (H) x 2 mm (t) (total 5mm)	10/pk

4. Components

Product name	Major components
e-PAGEL HR	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 300 V, 150 mA)
- Electrophoresis buffer etc.

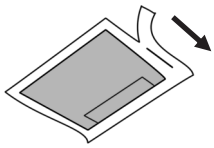
8. Precautions for use

- Please keep refrigerated. (5~10℃) Please do not place near the cold air outlet. It may freeze.
- Store the outer box upright according to the  mark. If not, the quality to deteriorate.
- Follow the  mark and store the gel with the well facing down. Please note that storing in the wrong direction will cause the quality to deteriorate.
- 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

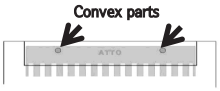
9-1. Preparation of gel and electrophoresis buffer

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

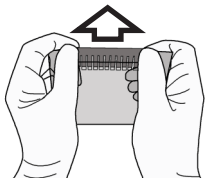


※If it is difficult to cut, use scissors.
※If you pull out the gel forcibly, the gel may peel off from the glass.

2. Pull out the comb slowly.



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



3. Prepare the electrophoresis buffer corresponding to the sample treatment (with or without SDS treatment) and the gel type.

SDS treated protein : EzRun (Tris/Gly/SDS)
: EzRun MOPS (Tris/MOPS/SDS)

SDS-free protein and nucleic acid
: EzRun TG (Tris/Gly)

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.

Comb (Number of wells)	Well size	Maximum applied amount
14 wells	4.2(W)×10(H) mm	24 μL
18 wells	2.9(W)×10(H) mm	18 μL
2-D	78(W)×25(H) mm	agarGEL 1 ea

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

Electrophoresis buffer		Voltage	Current	Time
EzRun Tris/Glycine/SDS	C.V.	300 V C.V.	Starting: 5 ~ 85 mA Ending: 25 ~ 45 mA	30-40 min
	C.V.	150 V, C.V.	Starting: 30 ~ 40 mA Ending: 10 ~ 20 mA	70-80 min
	C.C.	Starting: 75 ~ 95 V Ending: 180 ~ 220 V	20 mA/gel C.C.	75-85 min
EzRun MOPS Tris/MOPS/SDS	C.V.	250 V C.V.	Starting: 70 ~ 100 mA Ending: 30 ~ 50 mA	25-35 min
	C.V.	150 V C.V.	Starting: 40 ~ 60 mA Ending: 15 ~ 30 mA	50-60 min
	C.C.	Starting: 65 ~ 85 V Ending: 130 ~ 180 V	20 mA/gel C.C.	75-85 min
EzRun TG (Nucleic acid) Tris/Glycine	C.C.	Starting: 100 ~ 120 V Ending: 230 ~ 250 V	20mA/gel C.C.	60-90 min

※C.C.; Constant Current, C.V.; ConstantVoltage
※In case of constant current (constant voltage), set the voltage value (current value) with a margin.

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

※For constant voltage setting, please set it to 150 V or 300 V regardless of the number of gels. For constant current setting, double the current value according to the number of gels. For example, set to 40 mA for two gels.

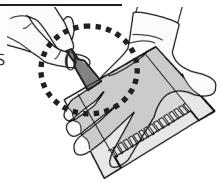
4. Apply the power and perform the electrophoresis.

※After electrophoresis, buffer solution can be hot. In case of 20 mA/gel constant current setting or 150 V constant voltage setting, it may increase to around 30℃(Depending on the number of gels). In case of 300 V constant voltage setting, it may increase to 35~50℃.

9-3. End of electrophoresis

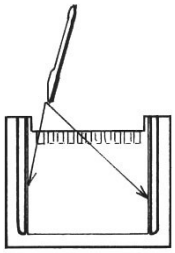
1. Stop migration when the dye front reaches the 5~10 mm upper position from the lower end of the gel.

※Please stop the operation according to the instruction manual attached to the electrophoresis device.



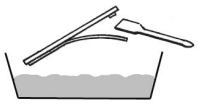
2. Remove the gel plate from the electrophoresis tank, insert a flat object such as a spatula between the glass, and gently move it up and down to open the gel plate. Remove the top glass plate.
3. Cut between the gel and the spacer with a spatula or scalpel.

※Moist spatula moves smoothly and prevents damage to the gel.



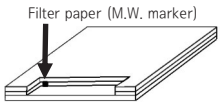
4. Transfer the gel to the vat containing the staining solution. Hold the gel plate with the gel side down, insert a spatula between the gel and the gel plate, and peel off the gel.
5. Slightly shake the vat to immerse the whole gel.

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



9-4. 2-D electrophoresis

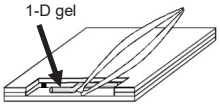
1. Dip molecular weight marker (~3 μL) on 3 mm × 3 mm filter paper. Place it on the edge of the gel in contact with the upper surface of the 2-D gel.
2. Place the equilibrated 1-D gel in contact with the upper surface of the 2-D gel.



※Adhere the 1-D and 2-D gels closely. A gap between the gels may cause the electrophoresis to be disturbed.

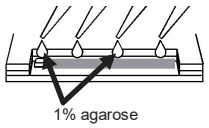
3. Drop 1% agarose solution and attach the 1-D and 2-D gels.

※Please use the agarose solution after cooling to around 60℃.



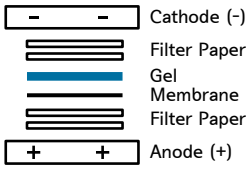
4. Perform electrophoresis (see section **9-2**).

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



9-5. Western blotting

1. Hydrophilize the PVDF membrane with methanol and equilibrate the membrane with blotting buffer. Immerse the filter paper in the blotting buffer.
2. Wash the electrophoresed gel with blotting buffer.
3. Refer to the figure on the right, place the filter paper, gel and PVDF membrane on top of each other and set in the blotting device.
4. Remove excess transfer buffer and bubble with a roller.
5. Refer to the table below and perform transfer.



		Filter paper	Voltage	Current	Time
Standard	C. C.	2~3 sheets x 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 HMW: 15~30 min
	C. V.		20V	0.5 A/gel	

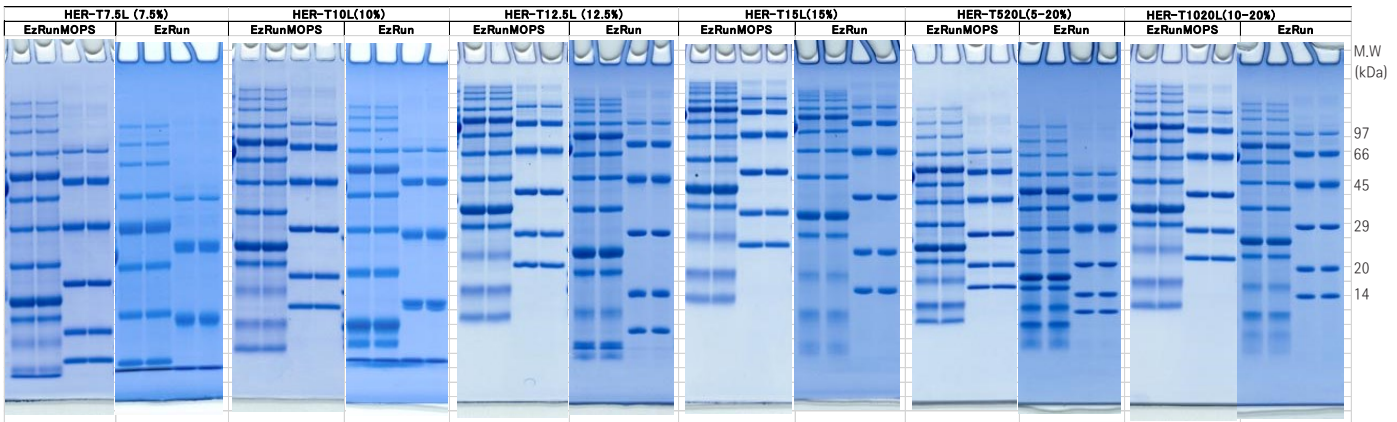
10. Reference

10-1. Gel concentration and separation range

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

Gel conc.	M.W. range (Protein)	M.W. range (Nucleic acid)
7.5%	40-400 kDa	200-3000 bp
10%	20-300 kDa	100-2000 bp
12.5%	10-250 kDa	70-1800 bp
15%	2-200 kDa	50-1500 bp
5-20%	5-400 kDa	30-2500 bp
10-20%	2-300 kDa	30-2000 bp

10-2. Electrophoresis pattern



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