e-PAGEL HR Instruction Manual

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

"e-Pagel HR" is a pre-cast polyacrylamide gel for protein and nucleic acid electrophoresis. Please use the electrophoresis system (for pre-cast gels) dedicated for ATTO mini-size gels.

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

3. Package

Name	Main component
e-PAGEL HR	Polyacrylamide gel

4. Components

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 and avoid freezing. 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

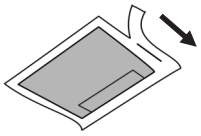
- Refrigeration at 5-10℃ is recommended for storage. It may freeze even in the refrigerator compartment, so keep it away from the cold air outlet of the refrigerator.
- The outer box should be stored upright according to the UP symbol. Please note that storing the product without standing it upright may cause deterioration in quality.
- Store the gel with the wells facing down, following the UP symbol. Please note that storing in the wrong way may cause quality deterioration.
- 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

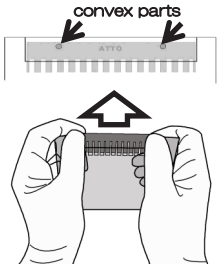
1. Open the package and take out the gel.

   ∗ 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 appropriate electrode buffer depending on the sample (Native or SDS-denatured) and gel type.

   SDS-PAGE: AE-1410 Ez Run (Tris/Gly/SDS), WSE-7065 Ez Run-MOPS (Tris/MOPS/SDS)

   Native-PAGE: WSE-7055 EZ Run TG (Tris/Gly), WSE-7056 EzRun ClearNative, WSE-7057 EzRun BlueNative

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.

   ∗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.

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
2-D	78(W)×25(H) mm	Agar GEL 1pc

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

Electrode buffer		Voltage	Current	Time
EzRun Tris/Glycine/SDS	C.V.	**300V setting**	At start：65-85mA At end：25-45mA	30-40 min
	C.V.	**150V setting**	At start：30-40mA At end：10-20mA	70-80 min
	C.C.	At start：75-95V At end：180-220V	**20mA/gel setting**	75-85 min
EzRun MOPS Tris/MOPS/SDS	C.V.	**250V setting**	At start：70-100mA At end：30-50mA	25-35 min
	C.V.	**150V setting**	At start：40-60mA At end：15-30mA	50-60 min
	C.C.	At start：65-85V At end：130-180V	**20mA/gel setting**	75-85 min
EzRun TG (Nucleic acid) Tris/Glycine	C.C.	At start：100-120V At end：230-250V	**20mA/gel setting**	60-90 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 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 150V or 300V regardless of the quantity of gels.

＊For constant current setting, multiply the current value by the quantity of gels. For example, if you have 2 gels, set the current value to 40 mA.

4. Turn on the power supply and start electrophoresis.

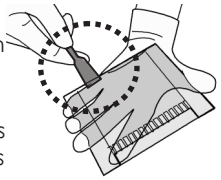
＊Please note that the temperature of the electrode buffer may rise during running. Depending on the number of gels, running conditions of 150 V c.v. or 20 mA/gel c.c. may raise the buffer temperature to around 30 °C, while running conditions of 300 V c.v. may raise the buffer temperature to 35-50 °C.

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 supply and stop running.

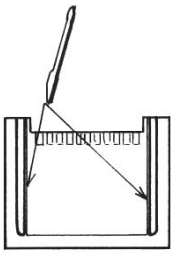
＊Please complete the electrophoresis operation according to the instruction manual attached to the electrophoresis device.

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 incisions between the gel and the side of the glass spacers.

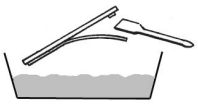
＊Keeping the spatula wet with buffer will prevent the gel from sticking or tearing.



4. Transfer the gel to a container filled with staining solution etc. 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.

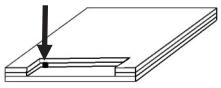
＊CBB staining, silver staining, reverse staining, fluorescent staining, etc. can be used.



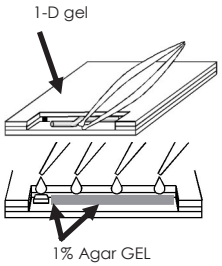
9-4. 2D electrophoresis

1. Soak a 3 mm x 3 mm filter paper with the molecular weight marker (~3 µL). Place it on the side edge of the gel to contact with the top side of the second dimension gel.

Filter paper
(molecular weight marker)



2. Place the equilibrated the first dimension (1D) gel on the top of the second dimension (2D) gel.



＊The 1D gel and the 2D gel should be in close contact with each other. Gaps between gels can cause poor migration.

3. The 1D and 2D gels are fixed together by dropping a 1% agarose solution.

＊Cool the agarose solution to around 60°C before use.

4. Perform electrophoresis (Please see Section **9-2**).

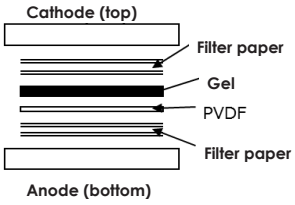
＊Follow the instruction manual attached to the agar gel and electrophoresis apparatus.

9-5. 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. Set power supply refer to the table below and start transferring.

		Filter paper	Voltage	Current	Time
Standard	c. c.	2—3 pcs × 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	

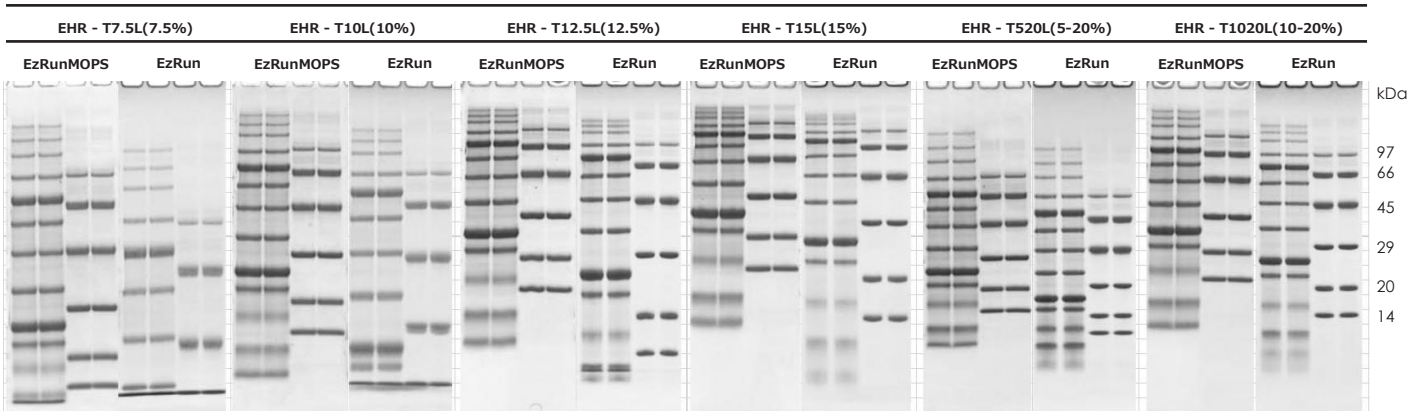
10. Reference

10-1. Gel concentration and molecular weight range of separation

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

Gel concentration	Molecular weight range of separation (Protein)	Molecular weight range of separation (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



ATTO Corporation
Head Office: 3-2-2 Moto-asakusa, Taito-ku, Tokyo 111-0041, JAPAN
TEL: +81-3-5827-4863 FAX: +81-3-5827-6647
E-mail: eig@atto.co.jp Website: http://www.atto.co.jp/eng/