WSE-7150 *EzGel Sep* Instruction Manual

24th May, 2016 Ver. 4

1. Safety precautions

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

2. Application purpose

The product is gel buffer used to make separating gel of polyacrylamide gel for protein electrophoresis based on Laemmli* method. To make separating gel of polyacrylamide gel, mix the product with Acrylamide/Bis, polymerization initiator (APS) and polymerization promotor (TEMED).

*Laemmli UK (1970), *Nature* **227** (5259): 680–685

3. Package

Product name	Volume	Package
EzGel Sep	250 mL	1 bottle

4. Components

Product name	Major component
EzGel Sep	1.5M Tris-HCl/pH 8.8

The product doesn’ t include toxic material and deleterious substance decided by Poisonous and Deleterious Substances Control Act, or a notifiable material exceeding to regulated amount for exclusion decided by Industrial Safety and Health Law and PRTR Law. If you need (M)SDS, please contact our company.

5. Preservation method

- Keep the product at room temperature. To preserve it stably for a long time, we recommend preserving it in a refrigerator.
- The expiration date for use is written on the label.

6. Disposal method

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

7. Necessary things other than the product

- ***EzGel Stack***（stacking gel buffer）
- Acrylamide/Bis mixture
- APS （Ammonium peroxodisulfate）
- TEMED（N,N,N',N'-tetramethyl ethylene diamine）
- An electrophoresis apparatus
- A gel caster
- A power supply
- Buffer for electrophoresis etc.

8. Precautions for use

- Without diluting the product, add the amount which is 1/4 of a total amount. For example, 2.5 mL of the product is required for making 10 mL gel solution.
- The table of gel composition in this manual is a little different from one in an instruction manual of our electrophoresis apparatus. We recommend referring to the former rather than the later when you make gel.
- Keep the product at room temperature. To preserve it stably for a long time, we recommend preserving it in a refrigerator.
- Use ***EzGel Stack*** for making stacking gel.

9. Usage

9-1. Reagent preparation

① **Acrylamide/Bis solution**

Acrylamide/Bis mixture whose cross-linked rate is 19:1, 29:1 or 37.5:1 is used generally depending on range of molecular weight cutoff. If cross-linked rate of the solution is high, fractionation range at the side of low molecular weight spreads. On the contrary, if cross-linked rate of the solution is low, fractionation range at the side of high molecular weight spreads. Refer to the following for preparing the solution.

30(w/v)% Acrylamide/Bis (29:1) solution

Weigh 29g of Acrylamide and 1g of N,N'-methylenebis (Acrylamide) , and dissolve them with 50 mL distilled water. After dissolving, dilute it in measuring cylinder to 100 mL total with distilled water.

30(w/v)% Acrylamide/Bis (37.5:1) solution

Weigh 29.22g of Acrylamide and 0.78g of N,N'-methylenebis (Acrylamide), dissolve them with 50 mL distilled water. After dissolving, dilute it in measuring cylinder to 100 mL total with distilled water.

※Monomer of Acrylamide has neurotoxicity. Protect your body with gloves etc. from it.

※30(w/v)% Acrylamide/Bis solution whose cross-linked rate is 29:1 or 37.5:1 is commercially available. If cross-linked rate of the solution is high (ratio of Bis is high), cross-linked structure is increased and hard gel whose grid structure is dense is made. Also, fractionation of low molecular weight range becomes clear.

② **10% APS （Ammonium peroxodisulfate）**

Weigh 0.1g of APS and dissolve it with 1 mL distilled water.

※Activity of 10% APS is decreased little by little, so that we recommend preparing it just before operation. It is storable for about 1 week in a refrigerator.

③ **TEMED（N,N,N',N'-tetramethyl ethylene diamine）**

Use it without diluting.

④ **Electrode buffer**

Prepare electrode buffer including SDS for SDS-PAGE. If samples don't include SDS, it is not necessary to include SDS in electrode buffer.

9-2. Gel casting

1. Refer to the composition table of gel (back of this page). Mix the product, Acrylamide/Bis solution and distilled water to make separating gel solution.

※Mix all solution except APS and TEMED.
※To make stacking gel, use ***EzGel Stack.***

2. Add APS and TEMED to separating solution in accordance with the composition table, and mix them without making bubbles.

3. Pour separating gel solution into assembled plates quickly.
※To confirm the usage of electrophoresis chamber and gel caster, contact the maker.

4. Overlay separating gel solution with distilled water (0.2～1.0 mL), without disturbing the interface.
※It is hard to polymerize Acrylamide with oxygen, so that distilled water is needed to cut off it.

5. Keep it calmly at room temperature for more than 30 minutes to polymerize separating gel.
※Confirm if the interface is clearly visible. Polymerizing time is varied depending on season and room temperature. Take note that it is hard to polymerize gel under 20℃.

6. Use paper towels to remove overlaid distilled water completely.

7. Refer to the composition table. Add APS and TEMED to stacking gel solution, and mix them without making bubbles.

8. Overlay separating gel with stacking gel solution quickly, and insert a comb.

9. Keep it calmly at room temperature for more than 30 minutes to polymerize stacking gel.

9-3. Electrophoresis

1. Prepare electrode buffer. The following buffer is available.
For Protein：***EzRun*** (Tris/Glycine/SDS), ***EzRun MOPS***, ***EzRun TG*** etc.

2. Set the gel in an electrophoresis apparatus, and connect to power supply.

3. Run the gel.

	Electrode buffer	Voltage	Mini gel	Compact gel
Protein	***EzRun***	150V	75～80 min	25～30 min
	EzRun MOPS	250V	20～25 min	10～15 min

《Recommended condition》・・・Running time is only a guide.

10. Composition table of gel

Use ***EzGel Sep*** for making separating gel, ***EzGel Stack*** for making stacking gel.

	Separating gel (10 mL)					Stacking gel
Gel concentration	5%	7.5%	10%	12.5%	15%	4.5%
Distilled water	5.8 mL	5 mL	4.2 mL	3.3 mL	2.5 mL	3 mL
30% Acrylamide/Bis solution	1.7 mL	2.5 mL	3.3 mL	4.2 mL	5 mL	0.75 mL
Gel buffer ***EzGel Sep***	2.5 mL	2.5 mL	2.5 mL	2.5 mL	2.5 mL	-
Gel buffer ***EzGel Stack***	-	-	-	-	-	1.25 mL
10% APS	0.075 mL	0.075 mL	0.05 mL	0.05 mL	0.05 mL	0.05 mL
TEMED	0.005 mL	0.005 mL	0.005 mL	0.005 mL	0.005 mL	0.0025 mL

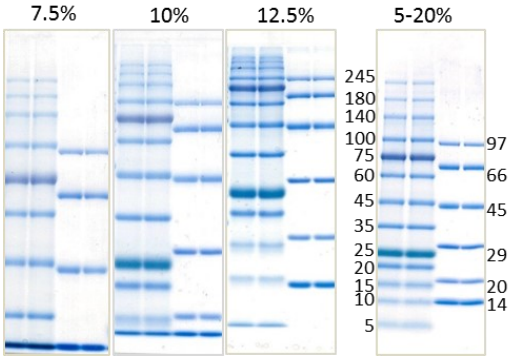
※The required amount for 1 gel of Mini gel size.
※It is unnecessary to add SDS.

Gel concentration and Fractionation range

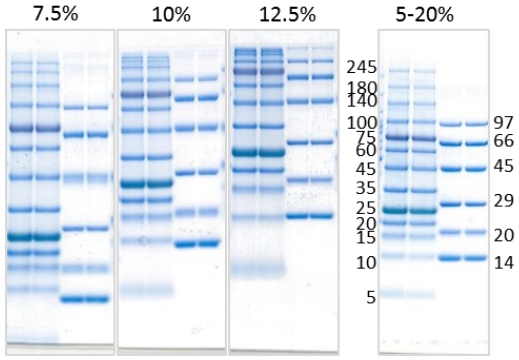
Gel concentration	Fractionation range (Protein)	Fractionation range (Nucleic acid)
5%	80~400 k Da	
7.5%	40~200 k Da	750~3000 bp
10%	20~130 k Da	150~2000 bp
12.5%	14~80 kDa	70~1800 bp
15%	10~60 kDa	50~1500 bp

※In the case of using 30(W/V)% Acrylamide/Bis (37.5:1) solution

11. Electrophoresis pattern



In the case of ***EzRun*** (Tris/Glycine/SDS) buffer



In the case of ***EzRun MOPS*** buffer

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