WSE-7310 *EzGel Ace* Instruction Manual

August 15th, 2016 Ver.4

1. Safety precautions

Before using this product, read this instruction manual thoroughly at first. Do not start the operation until you understand the contents of the manual. Also, this document describes only method used for specified purpose with this product. Do not use this product for any purpose or by any method other than described here. If you use this product for any purpose or by any method other than described in this manual, you will be held responsible for any necessary safety measures as operator. Additionally, read a manual of the equipment you use simultaneously and understand how to use and specification.

2. Application

This product is a gel buffer for making a polyacrylamide gel for protein and nucleic acid electrophoresis. A polyacrylamide gel is made by mixing this product, Acrylamide/Bis, polymerization initiator (APS) and polymerization promotor (TEMED). A gel made of this product is a neutral gel, so it can be preserved for a long time in refrigerator.

3. Package

Product name	Volume	Package
EzGel Ace	250 mL	1 bottle

4. Components

Product name	Main component
EzGel Ace	Tris

This product doesn't include poison and deleterious substance decided by Poisonous and Deleterious Substances Control Act, and notifiable chemical substances exceeding to regulated amount for excluding decided by PRTR Law and Industrial Safety and Health Law. If you need SDS, please contact our sales department.

5. Preservation method

- Keep this product at room temperature. Saving it in refrigerator is recommendable for keeping it stable for a long time.
- Expiration date is described in a bottle label.

6. Disposal method

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

7. Necessary things other than this product

- Acrylamide/Bis liquid mixture
- APS (Ammonium persulfate)
- TEMED (N,N,N',N'-Tetramethyl ethylene diamine)
- Electrophoresis apparatus
- Gel cast kit
- Power supply
- Electrode buffer etc.

8. Precaution for use

- Amount needed of stock solution is a quarter of whole amount of gel solution. For example, in the case of making 10mL gel solution, 2.5mL stock solution of this product is needed to add.
- Gel composition table of this document is a bit different from one described in an instruction manual of ATTO products such as electrophoresis apparatus etc. If you use this product, we recommend you to make a gel in accordance with the table described in this document.
- Keep this product at room temperature. Saving it in refrigerator is recommendable for keeping it stable for a long time.

9. Usage

9-1. Preparation of reagent

① Acrylamide/Bis solution

In general, Acrylamide/Bis liquid mixture of which cross-linked rate is 19:1, 29:1, 37.5:1 is used depending on a range of molecular cutoff. The higher cross-linked rate of solution is, the more fractionation range of low molecular weight side spreads. Also the lower cross-linked rate of solution is, the more fractionation range of high molecular weight side spreads. Refer to the below examples and prepare reagent depending on targeted fractionation range.

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

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

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

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

※A monomer of Acrylamide is neurotoxic. When you use it, put on gloves and so on for protecting your body.

※30(w/v)% Acrylamide/Bis solution (29:1), 30(w/v)% Acrylamide/Bis solution (37.5:1) and so on are commercially available. If cross-linked rate is high (a proportion of Bis is large), firm gel whose grid structure has high density is made and fractionation of low molecular weight range becomes more clear.

② 10% APS (Ammonium persulfate)

Prepare 0.1g APS and dissolve it in 1mL distilled water.

※10% APS is devitalized little by little, so we recommend preparation before use. It can be preserved for about 1 week in refrigerator.

③ TEMED (N,N,N',N'-Tetramethyl ethylene diamine)

Stock solution is used as is.

④ Electrode buffer for electrophoresis chamber

Prepare electrode buffer including SDS for SDS-PAGE. If a sample is not SDS-treated, electrode buffer also doesn't need SDS.

9-2. Gel casting

1. Refer to Gel composition table, and mix this product, Acrylamide/Bis solution and distilled water for making gel solution of separation gel and concentrated gel.

※Mix solution except APS and TEMED. This gel solution can be preserved stably for about 1month in refrigerator. When the preserved gel solution is used, add APS and TEMED appropriately. Concentrated gel can be made from gel solution for separation gel diluted by distilled water (It doesn't affect electrophoresis even though concentration of gel buffer for concentrated gel is dilute).

2. Refer to Gel composition table, and add APS and TEMED to separation gel solution. Mix them so as not to be bubbled.

3. Pour separation gel solution into assembled electrophoresis plates quickly.

※Contact makers of electrophoresis chamber and gel cast kit to confirm their usage.

4. Put distilled water (0.2-1.0mL) in so as not to disturb the boundary surface.

※Put distilled water in to prevent from oxygen because it is difficult for Acrylamide to polymerize if there is oxygen.

5. Leave it quietly at room temperature for more than 30 min to polymerize separation gel.

※Confirm that a boundary surface is clearly visible. Polymerization time varies depending on season and room temperature. Lower than 20℃ is not suitable for polymerization.

6. Remove put distilled water completely with paper towel.

7. Refer to Gel composition table, and add APS and TEMED to concentrated gel solution. Mix them so as not to be bubbled.

8. Put concentrated gel on the separation gel quickly, and insert a comb.

9. Leave it quietly at room temperature for more than 30min to polymerize concentrated gel.

※If you don't make concentrated gel, pour separation gel solution to the edge of electrophoresis plates. After inserting a comb, if you put a small amount of distilled water (several tens μL) in, a gel is made well.

9-3. Electrophoresis

1. Prepare electrode buffer. ATTO electrode buffer series are also available for electrophoresis.

   Protein : ***EzRun*** (Tris/Glycine/SDS), ***EzRun MOPS***, ***EzRun TG*** etc.

   Nucleic acid : ***EzRun TG*** etc.

2. Set a gel to electrophoresis apparatus and connect it to a power supply.

3. Apply an electrical current to electrophorese.

	Electrode buffer	Condition	Mini gel	Compact gel
Protein	***EzRun***	300V	30～35 min	10～15 min
	EzRun MOPS	250V	20～25 min	10～15 min
Nucleic acid	***EzRun TG***	20mA/gel	60 min	30 min

《Required condition》・・・Migration time is approximate.

10. Gel composition table

	Separation gel (10mL)					Concentrated 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 (***EzGelAce***)	2.5 mL	2.5 mL	2.5 mL	2.5 mL	2.5 mL	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

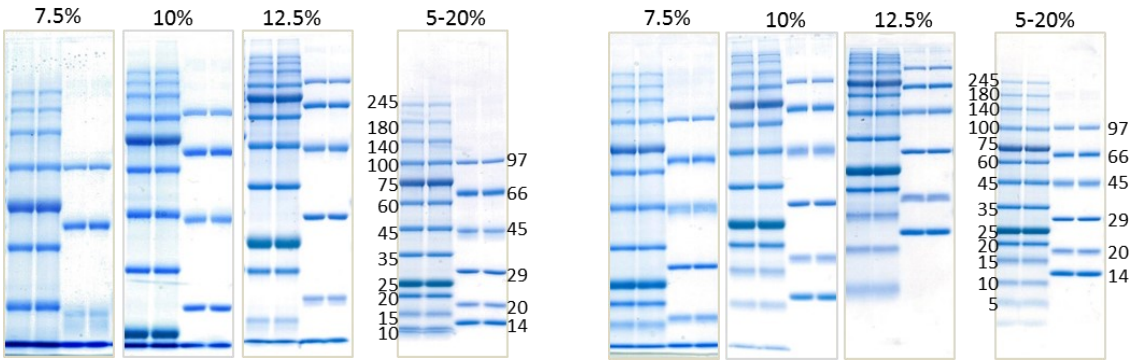
※Necessary amount for 1 mini gel.

Gel concentration and fractionation range

Gel concentration	Fractionation range (protein)	Fractionation range (nucleic acid)
5%	80~400 kDa	
7.5%	40~200 kDa	750~3000 bp
10%	20~130 kDa	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



With ***EzRun*** (Tris/Glycine/SDS) electrode buffer With ***EzRun MOPS*** electrode buffer

ATTO CORPORATION
3-2-2 Motoasakusa,
Taito-ku, Tokyo 111-0041, JAPAN
Tel +81 3 5827 4863
Fax +81 3 5827 6647
E-mail: eig@atto.co.jp
http://www.atto.co.jp/eng

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