ATTO

A- C310 / 38 / 510 / 58
A-M310 / 38 / 510 / 58

Agar GEL Instruction Manual

Version13 October 1, 2019

1. Safety warnings and precautions

Correct operations are necessary Safety warnings and precautions of this product. The complete instructions should be read and fully understood before attempting to use 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 is prohibited. User shall be liable for all safety measures needed for any use other than specified in the manual. Please read carefully and understand not only this instruction manual but also the instruction manuals for "Disk Run" and "Mini Compact Disc Electrophoresis System".

2. Introduction

Agar GEL is a precast agarose gel for isoelectric focusing of proteins by using Disc Run or Mini Compact Disc Electrophoresis System.

3. Package

Name	Quantity	Package
Agar GEL	5 column gels / pkg	2 packages (10 column gels)

Specification

Name	***Agar GEL*** （For compact size）		***Agar GEL*** （For mini size）	
Model	***A-C310/38/58/510***		***A-M310/38/58/510***	
Electrophoresis column	Size：Outer diameter φ7×75mm Material: Glass		Size：Outer diameter φ7×100mm Material: Glass	
Gel size	Outer diameter φ2.5×50mm		Outer diameter φ2.5×75mm	
Sample volume	Up to 150 µg, 120 µL Small volume recommended		Up to 300 µg, 120 µL Small volume recommended	
pH range	Common to compact type and mini type			
	A-C/M310 pH3-10	***A-C/M38*** pH3-8	***A-C/M510*** pH5-10	***A-C/M58*** pH5-8

4. Composition

Name	Component
Agar GEL	Agarose, ampholyte, urea, thiourea

This product includes notifiable materials exceeding to regulated amount for excluding decided by PRTR Law. Please contact our sales department about its MSDS.

5. Storage

- ***Agar GEL*** should be stored in a tightly closed and stored in dark at 5-10°C. The quality is stable until the expiration date (5 months from the date of manufacture) in an unopened package.
- Do not remove the anti-drying filter paper from the bag. After opening the package, do not discard the layered solution of unused gel and store it in the refrigerator with the zipper completely closed.
- Even if the layered solution flows out from the column during transportation, it can be used as long as a few mm of the layered solution remains on the gel. If the layered solution is completely lost and the gel dries, the column becomes unusable.

6. Disposal method

- For disposal of glass column and each reagent, strictly comply the instruction of your organization.
- Material of laminating zipper pack

  Polyethylene terephthalate / polypropylene

7. Items required in addition to this product

- The following reagents are not included in this product.

  Sodium hydroxide

  DL-aspartic acid (or phosphoric acid)

  Urea
- Isoelectric focusing electrophoresis system

  WSE-1510 Disc Gel EP Kit (discRun-R)

  AE-6540B Disc Gel EP Chamber
- 2D electrophoresis system

  WSE-1025 cPAGE Ace Twin (for compact size)

  WSE-1150 pageRunAce (for mini size)

  AE-6530 mPAGE Chamber (for mini size)
- Power supply

  AE-8155 myPowerII 500

  WSE-3100 PowerStation Ghibli I
- Shaker

  WSC-2400 SeesawShaker atto

8. Precautions for use

- Be sure to wear gloves when handling this product.
- A dialysis membrane is attached to the bottom end of the column. Use the column for electrophoresis without removing the dialysis membrane.

9. Usage

Details instructions for use are described in the instruction manual of "Disc Run" or "Mini Compact Disc Electrophoresis System" of Atto products. If you do not have it, please contact the distributor where you purchased the product.

A. Preparation of reagents

The following reagents are not included in the kit and should be prepared.

- Upper (Cathode) electrode solution：200 mM sodium hydroxide

  (Dissolve 4.0 g of sodium hydroxide in 0.5 L of DW.)

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Distributed by:

- Lower (Anode) electrode solution：40mM DL-aspartic acid

(Dissolve 5.32 g of DL-aspartic acid in 1 L of DW.)

Alternatively, 10 mM phosphoric acid can be used.

(Add 680 µL of phosphoric acid (85%) to 1 L of DW.)

- Layered solution: 2M urea

(Dissolve 1.2 g of urea in 10 mL of DW.)

B, Preparation of isoelectric focusing

1. Take out the Agar gel from the package.

① Wear clean gloves to prevent protein and dirt from your hands.

② Open the zipper and remove the number of gels to be used from the package. Do not remove the filter paper from the package as it is used to prevent drying.

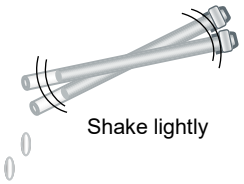
③ Store unused agar gels in a tightly closed and put it in a refrigerator. It will deteriorate if left at room temperature.

2. Remove the layered solution from the top of the gel (just before use).

① Invert the agar gel and shake it gently to discard the layered liquid.

*Please remove the layered solution just before use.

*If you shake too hard, the gel may be deformed or the glass may be broken. The top of the gel is 25 mm below the top of the glass column.

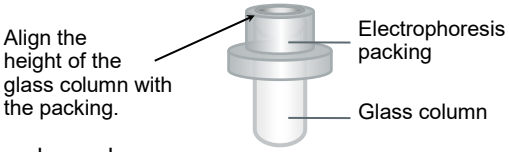


3. Set the column to the electrophoresis chamber.

① Attach the electrophoresis packing to the upper chamber of the Disc Run or Mini Compact Disc.

② Insert the side of the Agar gel without the dialysis membrane from the bottom of the packing.

③ Align the height of the top of the electrophoresis packing and the top of the glass column.



4. Load samples.

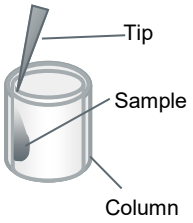
① Pour 550mL of the lower (Anode) electrode solution into the lower chamber.

② Place the upper chamber into the lower chamber.

③ Load the sample solution to the top of the Agar gel (50 µL or less, as little as possible). Please refer to the table below for the amount of protein to be loaded.

Detection method	Compact size	Mini size
CBB staining	100 µg	200 µg
Silver staining	1-2 µg	2-4 µg

∗The sample should be slowly layered using a 100 µL tip or even a thinner tip, and allowed to run down the inside of the glass column. If the sample is loaded rapidly, the sample solution may clog the inside of the glass column, causing air bubbles to form. Look at the electrophoresis column from directly above and check that there are no bubbles left in the glass column. If there are air bubbles, remove them with the tip of a micro syringe or a syringe needle.

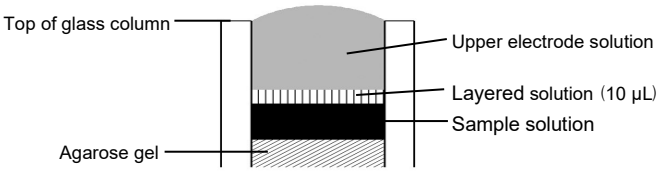


5. Load the layered solution and pour the upper electrode solution.

① Load 10 µL of the layered solution on the top of the sample solution.

② Load the upper electrode solution on top of this layered solution and fill it up to the top of the glass column.

∗Use a 100 µL tip or even a thinner tip and let it run down the inside of the glass column.



∗If the sample is loaded too quickly, the interface with the sample will be disturbed, which may cause electrophoresis failure.

C, Isoelectric focusing

1. Pour 40 mL of the upper (cathode) electrode solution into the upper chamber. Pour it gently so that the electrode solution does not hit to the top of the glass column directly.
2. At a constant voltage of 300V, it runs for 240 minutes for the mini size and 150 minutes for the compact size (Please confirm the optimum conditions by preliminary experiments).

∗Do not run at a voltage exceeding 300V from the start of energization as it may cause gel deformation. If the amount of sample is large or the amount of protein is increased, use the step setting that increases the voltage over time from a low voltage of about 50V.

D, End of electrophoresis

1. After the setting time is over, drain the upper electrode solution.

∗ Since it is an alkaline solution, be careful when handling it and be sure to protect yourself with gloves.

2. Remove the glass column from the upper tank, lay it horizontally, and remove the dialysis membrane.

∗If you tilt it diagonally, the gel will slide off.

3. After removing the dialysis membrane, gently tilt the glass column to collect the gel, and immerse it into a fixing solution, staining solution, etc.

∗For details and further operation, please follow the instruction manual of "Disc Run" and "Mini Compact Disc Electrophoresis System".