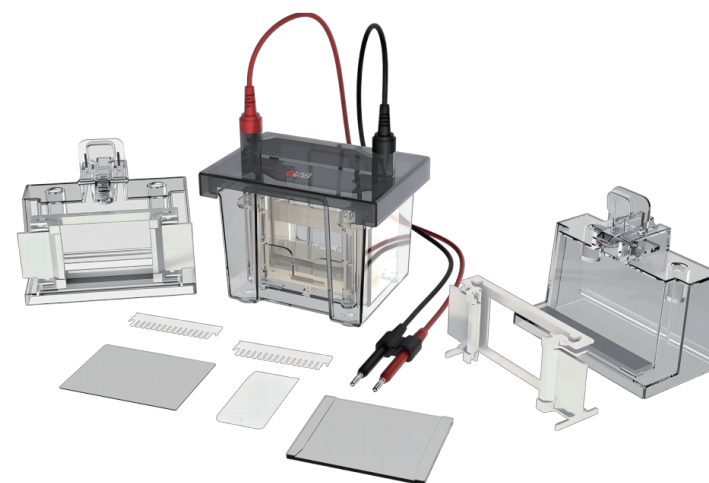

DL-MINI01 vertical electrophoresis tank

operating instruction



DLAB SCIENTIFIC CO.,LTD.
No.31, Yu'an Road, Beijing Airport Economic Core Zone, Shunyi
District, Beijing 101318, China
Tel: 86-10-85653451/52/53, 60498800
Fax: 86-10-85653383
E-mail: info@dlabsci.com
Service: service@dlabsci.com
Service Hotline: 400-1100-556
Web: www.dlabsci.com



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1.Basic product information

1.1 Product Introduction

The DL-MINI01 electrophoresis tank supports both manual gel preparation and precast gel solutions. The system features a gel plate holder with permanent adhesive gel spacer plates, streamlining manual casting processes while eliminating leakage risks. Capable of handling 1-2 gel layers, this compact tank is compatible with other large-scale electrophoresis equipment from Long Laboratories, making it ideal for tank-based electrophoresis and two-dimensional electrophoresis applications.

1.2 Component composition

To obtain optimal performance from the DL-MINI01 electrophoresis tank, assemble and disassemble the sample tank prior to use to familiarize yourself with the components listed in Tables 1 and 2 (see Figures 1 and 2).

Table 1.DL-MINI01 components.

Assembly	Remarks
Thick gelatinous glass plate	The thick glass is a higher glass plate, which is permanently attached with gel separator. The thickness of the separator is 1.0mm and 1.5mm, and these thicknesses are marked on each separatoron-board .
Gelatin glass sheet	Thin plates are shorter sheets of glass that combine with thick plates to form a gel box sandwich.
Clamping frame	When the sandwich frame is placed on the table, it can align and fix the thick plate and thin plate evenly together, thus forming a sandwich structure of gel box before making the glue.
Gel box assembly	A laminated frame, a thick plate and a thin plate form a gel box assembly.
Transparent glue seat	During the gelation process, this transparent gel base ensures that the gel cartridge assembly is secure. It contains a pressure lever that seals the gel cartridge assembly onto the rubber gasket.
Gel box laminates	A thick plate and a thin plate with a polymer gel form a gel sandwich.
Gel barrier	Use molded gel baffles when running one or three gels.
Electrode assembly	The electrode assembly carries the gel sandwich, which contains a gasket, upper and lower electrodes, and banana plugs for connections. The anode (lower electrode) banana plug is red Marking, cathode packaging (upper electrode) banana plugs are marked with black.

Table 2. DL-MINI01 Standard Configuration..

Assembly	Quantity	Remarks
Main body of electrophoresis tank	1 unit	Contains a cover, electrode, wire and buffer tank
Electrolysis tank core (main core and auxiliary core)	1 set	Used to fix the gel plate
Gelatinous glass plate (1.0 mm)	5 block	101 mm × 82 mm
Gelatin glass sheet	5 block	101 mm × 73 mm
10 tooth sampling comb (1.0 mm)	Two	Suitable for different sample sizes
15 tooth sampling comb (1.0 mm)	Two	Suitable for different sample sizes
Transparent glue seat	Two	Contains gray silicone rubber pad
Clamping frame	Two	Secure the glass panel
A glue shovel	One	Used for picking up glue
Gel barrier	One	Auxiliary glue preparation

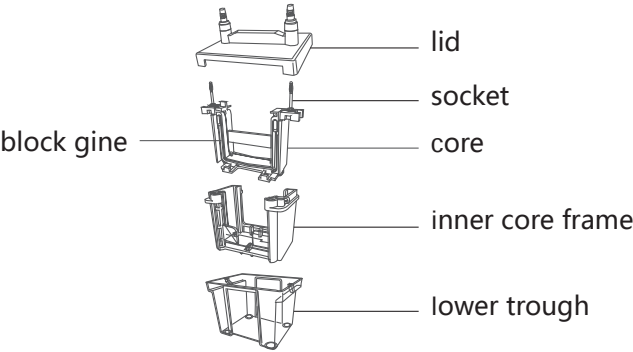


Figure 1. Assembling DL-MINI01

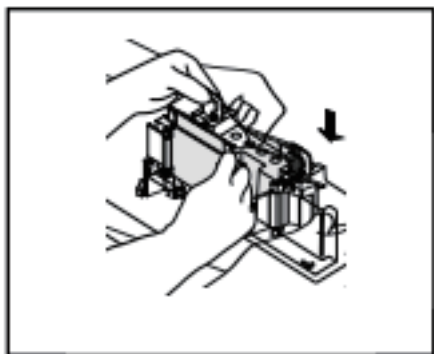


Figure 2. Assembling the DL-MINI01 clip frame and transparent glue seat.

1.3 Technical specifications

The specifications of DL-MINI01 are as shown in Tables 3 and 4.

Table 3. DL-MINI01 Specifications.

Project	Parameter
Number of gels	1-2 blocks (operational at the same time)
Gel size	Hand-injected glue: 8.3 cm × 7.3 cm; precast glue: 8.6 cm × 6.8 cm
Glass plate size	Thin glass plate: 10.1 cm × 7.3 cm; thick glass plate (with fixed edge strip): 10.1 cm × 8.2 cm
Gel thickness	0.75 mm, 1.0 mm, 1.5 mm (optional)
Number of comb teeth in the sampling device	10,15 gears (optional)
outline dimension	16*11.5*15cm
Buffer capacity	The inner tank is 130 mL; the outer tank is 750 mL
Electrode materials	99.99% high purity platinum wire, excellent electrical conductivity
peak voltage	600 V (DC)
maximum power	30 W

Table 4. Maximum Sample Volume per Well.

		Gel thickness		
hole count	Wide hole	0.75mm	1.0mm	1.5mm
5	12.7mm	20ul	105ul	160ul
9	5.08mm	33ul	44ul	66ul
10	3.35mm	33ul	44ul	66ul
15	6.2mm	20ul	26ul	40ul
IPG	6.2	—	420ul	730ul
Pre-treatment/2-D				
Reference aperture width	3.1mm	13ul	17ul	30ul
Sample holes	71.1mm	310ul	400ul	680ul

1.4 Safety instructions

The power supply for DL-MINI01 is provided by an external DC voltage source (not included). The output of this power supply must be isolated from the external ground to ensure that the DC voltage output is floating relative to the ground.

- Maximum voltage limit of 600 V DC
- Maximum power limit 30W
- Maximum ambient temperature limit 40°C

The current flowing into the electrophoresis tank enters the device through the lid assembly, which provides safety interlock for users. When the lid is removed, the current flowing into the electrophoresis tank is disrupted. Always turn off the power supply before removing the lid. Do not attempt to use an electrophoresis tank without a safety lid.

2. Installation and operation

2.1 Gel making step

1. Assembly of glass and glue bracket

Note: All glass plates should be kept clean and dry.

- a. Place the laminated frame vertically with the pressure cam in the open position and placed forward on a flat surface.

- b. Select the pad with the desired gel thickness and place a thin plate on it (see Figure 3A).
- c. Orient the thick plate so that the markings face upward. Then slide two glass plates into the gluing frame, keeping the shorter plate facing the front of the frame (the side with the pressure cam) (see Figure 3B).

Note: Ensure that both plates are placed flat on a horizontal surface and the labels on the thick plate are in the correct direction. If the plates are not aligned or the direction is incorrect, leakage may occur.

- d. After the glass plate is in place, start the pressure cam to clamp the glass in the glazing frame (see Figure 3C). Ensure that both plates are flush at the bottom.

- e. The laminated frame (with a locking pressure cam facing outward) is placed on the transparent glue seat, and the spring-loaded lever of the glue seat is combined with the thick plate (see Figure 3D), so that the laminated frame is placed into the transparent glue seat.

- f. Repeat steps 1A-E for other gels.

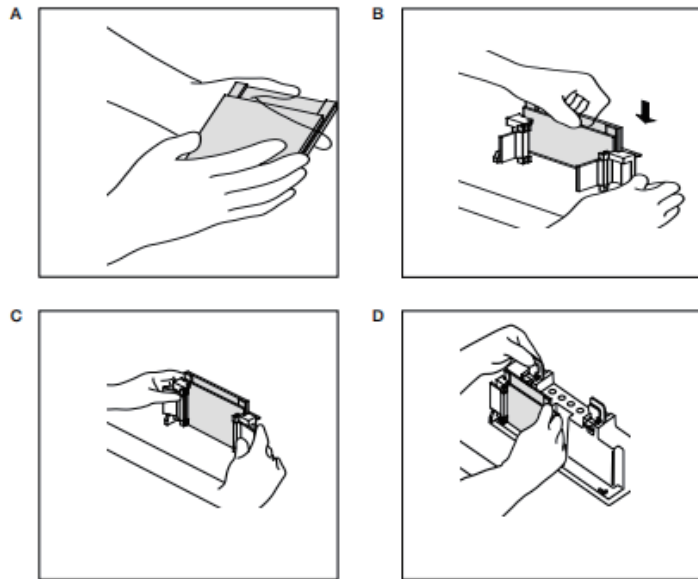


Figure 3. Assembling DL-MINI01 Glass and Gel-Making Bracket

2. Gel casting

a. Discontinuous polyacrylamide gel

- I. Place the comb completely into the assembled gel box. Mark the glass plate 1 cm below the comb teeth. This is the pouring level for separating the gel. Remove the comb.

- II. Prepare the separation gel solution by mixing all reagents except ammonium persulfate (APS) and TEMED. (Refer to Section 4 for the gel formulation.) De-gas the solution under vacuum for at least 15 minutes. Do not use a water bath pipette.

- III. Add APS and TEMED to the degassed solution, and pour the solution into the predetermined scale line using a glass or disposable plastic pipette. Keep the solution stable during operation to prevent mixing with air.

- IV. Immediately cover the solution with water or methanol.

Note: If water is used, add it slowly and evenly to prevent mixing.

- V. Perform gel polymerization for 45 minutes to 1 hour. Thoroughly rinse the gel surface with distilled water. Avoid leaving alcohol-coated gel on the surface for over 1 hour, as this may cause dehydration of the gel top. Note: The separated gel can be stored overnight at room temperature. Add 5 mL of 1:4 diluted 1.5 M Tris-HCl, pH 8.8 buffer (for Laemmli system) to the separated gel to maintain hydration. If using other buffer systems, add 5 mL of 1x separated gel buffer for storage.

- VI. Preparation of concentrated gel solution. Mix all reagents except APS and TEMED. Degas under vacuum for at least 15 minutes.

- VII. Dry the top of the separation gel with filter paper before pouring in the concentrate.

- VIII. Add APS and TEMED to the degassed concentrated gel solution and pour the solution on between the glass plates until reaching the top of the short plate.

a. Continuous polyacrylamide gel

- I. Prepare the solution by mixing all reagents except APS and TEMED. De-gas under vacuum for 15 minutes (see section 4 for the gel formulation).

- II. Add the APS and TEMED to a degassed solution, then pour the solution between the glass plates until reaching the top of the short plate.

- III. Starting from the top of the thick plate, insert the required comb between the pads, ensuring that the tongue at the end of each sampling comb is guided between the pads. Align the ridge of the sampling comb with the top of the thin plate and place it into the gel box.

- IV. Rinse the glue making bracket and leave it with distilled deionized water after use.

2.2 Module assembly

- Clean and dry the DL-MINI01 electrophoresis tank
- Electrophoresis module (for 1 or 2 gels, using electrode assembly module)
- Running buffer (750 mL for 1-2 gels)
- MInI-PROTEAN precast gel or hand poured gel
- Use the matching DL-MINI01 electrophoresis instrument

1. Assembly

- Set the inner core frame in the open position on a clean plane
- Position the first gel sandwich or gel cartridge (with the shorter end facing inward) on the gel holder, which is molded into the base of the inner frame assembly. Note that the gel will now tilt at a 30° angle toward the center of the holding frame. Handle the first gel carefully to ensure the holding frame remains balanced and doesn't tip over. Next, place the second gel on the opposite side of the frame, again positioning it against the holder. At this point, there will be two angled gel units: one on one side of the frame and the other on the opposite side, both tilting away from the center of the frame.

Note: It is crucial to place the gel cartridge in the holder with the short end facing inward. Additionally, the holder requires two gel cartridges to create a fully functional unit. If an odd number of gel cartridges are being used, a gel shield must be employed

- Gently pull the two gels together with one hand to ensure that they fit firmly and flatly against the strip embedded in the holding frame; at the same time, make sure that the short piece is just below the groove at the top of the strip.
- Gently press the gel sandwich or magnetic strip with one hand to reattach it to the backing (maintaining steady pressure to securely hold both gels in place), while sliding the arms of the clamping frame over the gel to lock them in position. Alternatively, you may choose to handle the entire assembly with both hands, ensuring the gel remains stable while simultaneously sliding the arms of the clamping frame into place.

The arms of the clamping frame push the short plates of each gel cartridge against the groove on the gel strip, creating a leak-proof seal (double-check to ensure the short plates are precisely positioned below the groove at the top of the gel strip). At this point, the sample wells can be rinsed with flow buffer and samples can be loaded.

Important note: Before attempting to lock the arms of the frame, make sure the gel box is fully aligned and stable with the groove on the module. To prevent gel displacement during locking, hold it firmly and evenly in the center of the module with one hand.

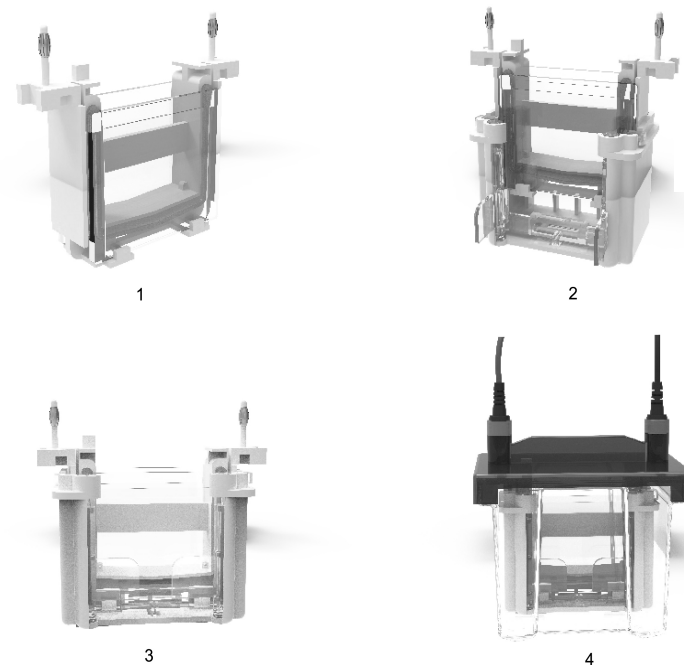


Figure 4. Assembling DL-MINI01 Electrophoresis Module

2. Sample loading

- Add buffer to the inner tank until the edge of the outer gel plate is reached.
 - When the sample is placed on a plane outside the tank, load the sample into each component.
 - Sample is loaded into the well using a microinjection or a pipette with a gel loading tip.
- Note: Load the sample slowly so that it settles evenly at the bottom of the well. Be careful not to puncture the bottom of the well with a syringe needle or pipette.
- Note: Samples may be loaded into the module before it is placed in the slot, and samples may also be loaded into the module after the module is placed in the slot. Both methods will produce acceptable results.

3. Placement instructions for electrode assembly in DL-MINI01 electrophoresis tank: For two gels, the total buffer volume is 750 ml.

a. First, place the electrophoretic outer tank on a flat surface with the front of the tank facing you. After proper alignment, the red mark on the top edge of the outer tank will be on your right side, while the black mark on the top edge of the outer tank will be on your left side.

b. Add buffer to the indicated level to fill the outer tank (lower shell).

4. DL-MINI01 electrophoresis tank assembly

Secure the cover onto the DL-MINI01 outer slot. Align the color-coded banana connector with the corresponding hole on the electrode assembly. This alignment is achieved by matching the cover's socket with the electrode component's connector. The stop block on the cover prevents misalignment. Note that the protruding strips on both sides of the outer slot now guide the cover into place. Gently press the cover with your thumb to ensure it fits snugly and securely against the outer slot.

5. Power supply conditions

a. Insert the wire into a suitable power supply with the correct polarity.

b. Apply the power supply to DL-MINI01 and start electrophoresis; it is recommended to use a constant voltage of 200 volts for SDS-PAGE and most in situ gel applications. The same voltage (200 volts) is used. The optimal voltage may vary depending on specific applications. For

SDS-PAGE, which runs at 200 volts for about 35 minutes.

Note: The electrophoresis time will vary from 35 to 45 minutes depending on the acrylamide content.

6. Gel removal

a. After electrophoresis, turn off the power supply and disconnect the connection.

b. Remove the top cover and carefully remove the electrode assembly. Pour off and discard the flow buffer.

Note: Before opening the arm of the component, be sure to drain the buffer to avoid overflow.

c. Open the arm of the component and remove the gel box.

d. Take the gel out of the gel box by gently separating the two plates of the gel box.

e. Remove the gel by flipping the gel and plate so that it floats in a fixative or transfer solution and stirring gently until the gel is separated from the plate.

f. Rinse the electrode assembly, laminated frame and outer tank of DL-MINI01 electrophoresis tank with distilled water after use.

3. Separation theory and optimization

3.1 Introduction

Polyacrylamide gel electrophoresis separates molecules in complex mixtures based on size and charge. During the process, complex interactions occur between the sample, gel matrix buffer, and electric current, resulting in band separation of individual molecules. Therefore, key variables to consider during electrophoresis include gel pore size, gel buffer system, and the properties of target molecules.

Gel permeability

The gel pores are formed through cross-linking between polyacrylamide and bisacrylamide to create a porous network. This structure enables molecular sieving through the gel matrix. The pore size of the gel is a function of the acrylamide monomer concentration (%T). By convention, polyacrylamide gels are characterized by %T, which refers to the weight percentage of total monomers including the crosslinking agent.

%T indicates the relative pore size of the gel. Generally, the pore size increases with Decreases with the increase of %T.

%T is calculated using the following formula.

$$\%T = \frac{(\text{g acrylamide} + \text{g linear crosslinker} \times 100\%)}{\text{total volume (ml)}}$$

%C is the ratio of acrylamide monomer in the solution of crosslinking agent and monomer. %C is calculated by the following formula.

$$C\% = \frac{\text{g crosslinking agent} \times 100\%}{\text{g acrylamide} + \text{g crosslinking agent}}$$

2.67% C is traditionally used for most analytical gels.

Gels can be prepared as a single continuous percentage throughout the gel or as a gradient %T through the gel. For single, percentage gels, typical components range from 7.5% to 20%, or a gradient range of 4-15 to 10-20%.

The optimal total monomer concentration for separation is termed the optimal %T. This optimal %T varies depending on the molecular weight of the target molecule. Empirically, the pore size that provides optimal resolution for proteins corresponds to a relative migration rate (Rf) value of 0.55-0.6. The Rf value for specific proteins is calculated as follows.

$$Rf = \frac{\text{distance of migration of a particular protein}}{\text{distance of migration of the ion frontier}}$$

Gel buffer system

The buffer system determines the required energy and affects the separation effect. The buffer system consists of the buffer solution used in the gel and the running buffer solution. The buffer system can be divided into continuous and discontinuous types.

Non-continuous buffer system

In discontinuous buffer systems, distinct buffer ions exist in both the gel and electrode solutions. By using different buffers in these solutions and adding overlay gels to the resolving gel, samples can be compressed into a thin starting band for precise analysis and separation of individual proteins. Although originally designed for undenatured (i.e., native) proteins, the most widely used discontinuous system is the SDS-PAGE buffer system proposed by Laemmli (1970). The formulation details are provided in Section 4.2.

3.2 SDS-PAGE (Laemmli) buffer system

The Laemmli buffer system is a discontinuous buffer containing sodium dodecyl sulfate (SDS). In this system, proteins are denatured by heating a buffer solution containing SDS and a thiol reducing agent (such as 2-mercaptoethanol). The resulting denatured peptides form rod-shaped structures with a uniform charge-to-mass ratio proportional to their molecular weights. This allows for protein separation based on molecular weight, making the system highly valuable for molecular quantification.

3.3 Non-denaturing polyacrylamide gel electrophoresis (Native PAGE)

Native PAGE (Native Polyacrylamide Gel Electrophoresis) is a technique for separating bioactive proteins. Unlike SDS-PAGE, protein migration in Native PAGE depends on both molecular size and charge. Currently, no single electrophoresis buffer system can optimize the separation of all natural proteins. Key parameters for protein separation in Native PAGE include the isoelectric point (pI) of the target protein and the pH value of the electrophoresis buffer.

pH take part in pI

The pH of electrophoresis buffer must be within the range that stabilizes target proteins while preserving their biological activity. Additionally, the buffer's pH should provide sufficient charge to enable protein migration through the gel. pH variations affect both the charge and size (hydrodynamic volume) of target proteins, thereby influencing migration rates. For example: when the buffer pH exceeds the protein's isoelectric point (pI), the protein becomes positively charged and migrates toward the positive electrode (anode). Conversely, when the buffer pH is below pI, the protein carries a positive charge and migrates toward the negative electrode (cathode). At pH equal to pI, the protein remains uncharged and does not migrate under the electric field.

The migration rate of proteins is most susceptible to the influence of buffer pH. A buffer with a pH closer to the protein's isoelectric point (pI) provides optimal resolution but may result in longer running times. Conversely, buffers with a pH far from pI allow faster protein

migration but potentially lower resolution. Therefore, selecting an appropriate pH represents a trade-off between separation efficiency and processing speed.

How to choose a Native PAGE system

1. Non-continuous buffer system (Ornstein)

The discontinuous buffer system should be the preferred non-modified gel system. The advantage of the discontinuous system is that it uses concentrated gel to concentrate dilute protein samples, but concentration may also cause some proteins to aggregate and interfere with resolution. If protein aggregation occurs, the continuous buffer system should be used.

Note: The pH of Ornstein system separation gel is approximately 9.5, which may exceed the stability range of certain proteins and cause denaturation. Additionally, the isoelectric point (pI) of target proteins might be too close to or higher than the pH value of Ornstein buffer (9.5), potentially resulting in extremely low net charge or positive net charge, which could significantly reduce or even prevent migration toward the anode. For alternative discontinuous systems, refer to the article by Chrambach and Jovin (1983).

Note: It is best to know the pI of the target protein before choosing a buffer system.

2. Continuous buffer system

When discontinuous systems are inadvisable due to protein aggregation caused by concentration, continuous buffer systems should be employed. In these systems, the same buffer solution is used for both the upper and lower electrode chambers as well as the gel. Since no concentration occurs, the migration band width of proteins is at least equivalent to the sample volume, thus minimizing sample loading is essential. In continuous systems, protein mobility is determined by pH rather than through molecular sieve action in polyacrylamide gels. Therefore, most applications recommend using 6% polyacrylamide gels; for exceptionally large proteins, 4% or 5% gels may be used. McLellan (1982) described various continuous buffer systems operating within pH ranges from 3.8 to 10.2.

■ 4. Preparation of reagents and stock solutions

4.1 Volume required per gel

The volumes listed in Table 5 are required for full filling of the gel cartridge. The amount can be adjusted according to the application (with or without an electrophoretic comb

With or without concentration gel, etc.).

Table 5. Capacity requirements.

Viscosity thickness (mm)	Volume (ml)
0.5	2.8
0.75	4.2
1	5.6
1.5	8.4

Note: 10 mL of monomer solution is sufficient to stack any thickness of gel.

4.2 Concentrate

configuration 5% concentrate formulation

Ingredient	1 mL	2 mL	3 mL	4 mL	5 mL	6 mL	8 mL	10 mL
water (mL)	0.68	1.4	2.1	2.7	3.4	4.1	5.5	6.8
30% acrylamide (mL)	0.17	0.33	0.5	0.67	0.83	1	1.3	1.7
1.0 mol/L Tris (pH 6.8, mL)	0.13	0.25	0.38	0.5	0.63	0.75	1	1.25
10% SDS (mL)	0.01	0.02	0.03	0.04	0.05	0.06	0.08	0.1
10% ammonium persulfate (mL)	0.01	0.02	0.03	0.04	0.05	0.06	0.08	0.1
TEMED (mL)	0.001	0.002	0.003	0.004	0.005	0.006	0.008	0.01

4.3 Preparation of Separation Gel (Tris-glycine SDS system)

6% separation gel formulation

Ingredient	5 mL	10 mL	15 mL	20 mL	25 mL	30 mL	40 mL
water (mL)	2.6	5.3	7.9	10.6	13.2	15.9	21.2
30% acrylamide (mL)	1	2	3	4	5	6	8
1.5 mol/L Tris (pH 8.8, mL)	1.3	2.5	3.8	5	6.3	7.5	10
10% SDS (mL)	0.05	0.1	0.15	0.2	0.25	0.3	0.4
10% ammonium persulfate (mL)	0.05	0.1	0.15	0.2	0.25	0.3	0.4
TEMED (mL)	0.004	0.008	0.012	0.016	0.02	0.024	0.032

8% Separation gel formulation

Ingredient	5 mL	10 mL	15 mL	20 mL	25 mL	30 mL	40 mL
water (mL)	2.3	4.6	6.9	9.3	11.5	13.9	18.5
30% acrylamide (mL)	1.3	2.7	4	5.3	6.7	8	10.7
1.5 mol/L Tris (pH 8.8, mL)	1.3	2.5	3.8	5	6.3	7.5	10
10% SDS (mL)	0.05	0.1	0.15	0.2	0.25	0.3	0.4
10% ammonium persulfate (mL)	0.05	0.1	0.15	0.2	0.25	0.3	0.4
TEMED (mL)	0.003	0.006	0.009	0.012	0.015	0.018	0.024

4.4 Common buffer and carrier buffer

Common buffer

Type of buffer solution	Fluid	Storage solution preparation
TAE (Tris-Acetate-EDTA)	1×	50×:242 g Tris, 57.1 mL glacial acetic acid, 100 mL 0.5 mol/L EDTA (pH 8.0)
TPE (Tris-Phosphate-EDTA)	1×	10×:108 g Tris, 15.5 ml 85% phosphoric acid, 40 ml 0.5 mol/L EDTA (pH 8.0)
TBE (Tris-Borate-EDTA)	0.5×	5 ×: 54 g Tris, 27.5 g boric acid, 20 ml 0.5 mol/L EDTA (pH 8.0)

6X sample buffer

Type	Ingredient	Storage temperature
I	0.25% bromophenol blue, 0.25% xylene cyan, 40% sucrose aqueous solutio	4℃
II	0.25% Boilurin, 0.25% xylene cyan, 15% sucrose water-soluble	room temperature
III	0.25% bromophenol blue, 0.25% xylene cyan, 30% glycerol aqueous solution	4℃
IV	0.25% Boilurubin, 40% sucrose aqueous solution	4℃

5. Maintenance

DL-MINI01 Electrophoresis tank and lid

After each use, thoroughly rinse the electrode assembly, accessories, cast seat and frame with distilled water.

Glass plates and combs

Clean with a laboratory detergent and thoroughly rinse with distilled water. Submerge the thick plate in a strong alkaline solution, such as>100 mM NaOH, for no more than 24 hours.
To maintain the service life of the adhesive, avoid prolonged exposure to cleaning solutions made with strong alkaline detergents (>5 days).

6. Troubleshooting Guide

Problem phenomena	Possible causes	Rx
The strips are in the shape of a "smile"	Inhomogeneous gel solidification (common in thick gels)	Extend the gel polymerization time to ensure full solidification
(Middle concave sides up)		
The strips are wrinkled	There are bubbles at the bottom of both plates	Remove the bottom bubble during assembly to ensure that the glass plate fits the gasket
(Down on both sides of the middle drum)		
Drag the bar	The sample was not dissolved well, the concentration of separation gel was too high, and the buffer solution failed	Centrifuge the samples before adding the sample, select an appropriate buffer to reduce the gel concentration, and so on
		Change the fresh buffer
The indicator belt runs out of the board	Buffer pH error or excessive gel concentration	Check the buffer solution formula and replace the correct pH buffer solution to reduce the gel concentration
Protein not separated		
The staining background is high	Impurities such as SDS are not completely removed	After electrophoresis, extend the washing time or increase the number of washing times with double distilled water
No bands after staining	The gel is too thick or the sample is insufficient	Reduce the gel thickness, increase the sample loading, and add BSA positive control

7. References

- Chrambach A and Jovin TM (1983) Selected buffer systems for moving boundary electrophoresis on gels at various pH values, presented in a simplified manner Electrophoresis 4, 190--204
- Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4 Nature 227, 680--685
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- Ornstein L (1964) Disc electrophoresis I Background and theory Ann N Y Acad Sci 121, 321--349

8.Quality assurance

The product complies with the laboratory electrophoresis equipment standard and provides a 5-year warranty (non-manual damage).
Please read the operation manual carefully before use to ensure correct use.
For inquiries or repair service requests, please contact Dalong service personnel.