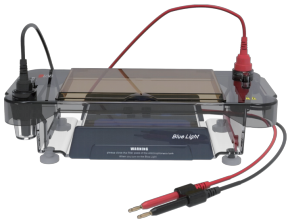
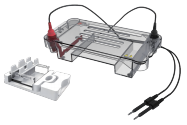
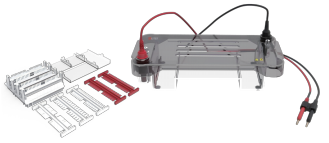
Multi functional horizontal

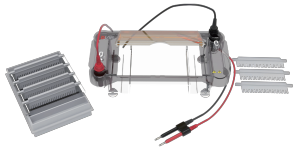
DL–Sub01,DL–Sub02, DL–Sub03 and DL–Sub03+

manual of operation









DLAB SCIENTIFIC CO.,LTD.
No.31, Yu'an Road, Beijing Airport Economic Core Zone, Shunyi District,Beijing101318,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

1.Basic information

1.1 Product Introduction

The DL-SUB series of instruments (DL-Sub01, DL-Sub02, DL-Sub03, and DL-Sub03+) are comprehensive and flexible gel electrophoresis systems designed for effective nucleic acid separation using underwater agarose gels. These gels are easy to dispense and provide excellent thermal conductivity. They allow layered sample placement while preventing electric field discontinuities caused by wick or sample well dehydration. Ideal for DNA restriction enzyme digestion products, PCR amplification fragments, and genomic/RNA isolation for Southern or Northern blotting analysis, underwater agarose gels can effectively separate nucleic acids ranging from 20 to 20 kilobases in length when properly operated.

The DL-SUB series is designed to deliver repeatable experimental results under rigorous conditions for years. These durable systems feature multiple characteristics that simplify and enhance the preparation and electrophoresis operations of agarose gels. The gel preparation device allows tape-free gel casting in trays. The replaceable electrode cartridge provides a convenient method for electrode wire replacement. Multiple base and tray configurations are available, including various preparation types

The analytical and comb compatible with the multi-channel pipette make these systems suitable for any agarose gel experiment.

1.2 Safety

The design of DL-SUB electrophoresis system maximizes user safety. The buffer tank is made of injection molding acrylic material to prevent user from electric shock.

Before use, inspect the base for cracks or gaps that could allow buffer fluid to leak out, posing potential electrical hazards. Additionally, check all cables, banana plugs, and standard connectors for loose connections, cracks, breaks, or corrosion. Never use any components with cracks, burnt-out parts, or corrosion – these may also create electrical risks.

During electrophoresis, check for signs of buffer leakage in the base and worktable. If buffer leakage is detected, immediately cut off the power supply of the electrophoresis tank.

The power supply of the DL-SUB system is provided by an external DC voltage source. The power supply must be grounded and isolated to enable DC voltage transmission

The output is in a relatively suspended state.

1.3 Environmental requirements

DL-Sub01, DL-Sub02 and DL-Sub03 and 03+ systems are designed to operate safely under the environmental conditions listed in Table 1.

Table 1. Environmental requirements

Parameter	Specifications
environment	For indoor use only
operational altitude	The altitude can reach more than 2000 meters
working temperature	4-40℃
Transportation and storage temperature	20℃ to 60℃C
relative humidity	50-80% (non-condensable,
Main power supply voltage fluctuation	0.5% (unless otherwise stated,
Overvoltage category	Ⅱ

* Operating the instrument outside this temperature range may not meet the performance specifications. The room temperature of 4-40°C (39-104°F) is considered safe.

* Store and transport the instrument in its shipping case to meet these temperature conditions.

Current from external power sources enters the battery unit through the cover assembly, which provides a safety interlock mechanism. When the cover is removed, the current flowing into the battery unit will be interrupted. Do not attempt to bypass this safety interlock. Always turn off the power supply before removing the cover or performing any operations on the battery unit.

1.4 Component composition

Each system in the DL-SUB series is equipped with components listed in Table 2. Please check the instrument to ensure all items are complete. Pay attention to any possible damage to the instrument during transportation. If any items are missing or damaged, please notify Dalong Xingchuang Experimental Instrument (Beijing) Co., LTD.

Table 2. System components

Name of accessory	Sub01	Sub02	Sub03,03+
	Quantity		
Cover of main slot (including wires)	One	One	One
Main body slot down (including electrode)	One	One	One
		Four	Two
Gel trays	One	Large rubber tray (120*120)	Large rubber tray (130*200)
		Wide rubber tray (120*60)	Small rubber tray (130*150)
		Long rubber tray (60*120)	
		Small rubber tray (60*60)	
A glue applicator	One	One	One
	Eight	7	Eight
Tooth comb	1.0mm sampling comb with 6 teeth (2)	2.0mm sampling comb (2+3 teeth)	1.0mm sampling comb (14 teeth) (2 pieces)
	1.0mm sampling comb with 11 teeth (2)	1.0mm sampling comb (6+13 teeth)	1.0mm sampling comb (18 teeth) (2 pieces)
	1.5mm sampling comb with 6 teeth (2)	1.0mm sampling comb (8+18 teeth)	1.0mm sampling comb (26 teeth) (2 pieces)
	1.5mm sampling comb with 11 teeth (2)	1.0mm sampling comb (11+25 teeth) (4 pieces)	1.5mm sampling comb (18 teeth) (2 pieces)

Note: Sub03+ is equipped with a blue photoelectric swimming monitor
Blue LED wavelength 470nm
Cooperating with blue dye can timely monitor the electrophoresis process
5 minutes to automatically turn off blue light, leaving it on for a long time can affect the lifespan of LED chips

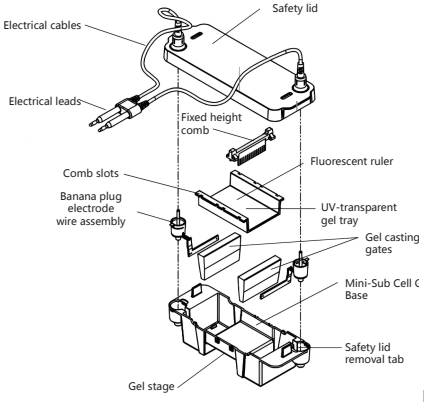


Figure 1. System components. This diagram shows the components in Sub01 system; Sub02 system and Sub03, Sub03+ systemsThe components are similar, but not identical.

1.5 Technical specifications

Table 3. Specifications

Project	Sub01	Sub02	Sub03,03+
	Parameter		
		Big glue 120 x 120 mm	Big glue 130×150mm
Gel area (width × length)	75×60 mm	Wide glue 120×60 mm	Small glue 130×200mm
		Long rubber 60×120 mm	
		Small glue 60×60 mm	
Number of teeth in sample	6 or 11 holes (optional)	2, 3, 6, 8, 11,13,18,25 (yes select)	1.0mm (14,18,26 teeth), 1.5mm (18 teeth)
outline dimension	220*100*100mm	330*160*120mm	340*170*130mm
Buffer volume	300 mL	700 mL	800mL
Maximum voltage load	500V	500V	500V
Applicable gel concentration range	0.8%-2.0% agarose gel	0.8%-2.0% agarose gel	0.8%-2.0% agarose gel
Electrode materials	99.99% high purity platinum wire, excellent electrical conductivity	99.99% high purity platinum wire, excellent electrical conductivity	99.99% high purity platinum wire, excellent electrical conductivity
operating temperature range	≤30℃ (operating)	30℃ (at runtime)	30℃ (at runtime)

* The volume of the base buffer in a Sub-type electrophoresis tank varies depending on the size and thickness of the gel used.

2. How to operate

Note: For information on RNA gel preparation, see section 3 "Preparation of Gel and Electrophoresis Reagents". For more information on DNA and RNA electrophoresis, see section 6 "References".

2.1 DNA gel preparation step

DNA agarose gels can be used to separate and observe DNA of various sizes. Before casting the agarose gel, consult Table 4 and determine the appropriate percentage of agarose gel based on the size of the DNA to be separated.

step

1. Determine the amount of agarose required (g) for the preparation of agarose gel with the required concentration and volume. Table 4 and Table 5 are used as reference for the requirements of agarose concentration and gel volume.

Table 4. Gel concentrations required for DNA isolation

Gel strength （%）	DNA big or small
0.5	1—30kb
0.75	800bp—10kb
1	500bp—10kb
1.25	400bp—7kb
1.5	200bp—3kb
2	100bp—2.5kb
3	40bp—2kb
4	4—400bp

* Please refer to the references for more information.

Table 5. Gel volume requirements

Gel size (thickness)	0.25cm	0.5ml	0.75ml	1.0ml
lampstand				
7×7cm	10ml	20ml	30ml	40ml
15×7cm	20ml	40ml	60ml	80ml
5×15cm	50ml	100ml	150ml	200ml
pallet				
7×7cm	10ml	20ml	30ml	40ml
7×10cm	15ml	30ml	45ml	60ml
15×7cm	20ml	40ml	60ml	80ml
15×10cm	30ml	60ml	90ml	120ml
15×15cm	50ml	100ml	150ml	200ml
15×20cm	70ml	140ml	210ml	280ml
15×25cm	90ml	180ml	270ml	360ml

2. Add agarose to a suitable container (e.g., 250 mL conical flask or Wheaton bottle). Add an appropriate amount of 1x electrophoresis buffer (see Section 3: Preparation of Gel and Electrophoresis Reagents for Buffer Preparation) and rotate to suspend the agarose powder in the buffer. If using a conical flask, invert the 25 mL conical flask containing agarose onto the open end of the 250 mL conical flask. A small flask serves as a reflux chamber, allowing prolonged or intense boiling without excessive evaporation.

Note: You can mark the same level as the liquid on the lower flask. If evaporation occurs, you can add water to restore the liquid to its original starting level.

3. Agarose can be melted by boiling on a magnetic hot plate (step 4a) or in a microwave oven (step 4b).

Note: Always wear protective gloves, safety goggles and lab coats when preparing and perfusing agarose gels. Contact with skin with hot agarose-containing blood vessels can cause severe burns. Additionally, molten agarose will boil when vortexed.

Maglev plate method

4a. Add a stir bar to the undissolved agarose solution. Heat the solution to boiling while stirring on a magnetic heating plate. Bubbles or foam should break before rising to the neck of the culture flask.

Microwave oven method

4b. Place the gel solution in a microwave oven. Set the timer to at least 5 minutes using a low to medium setting, and pause the microwave every 30 seconds. Gently rotate the culture flask to suspend any undissolved agarose. This technique is the fastest and safest method for dissolving agarose.

5. Boil the solution and rotate until all translucent agarose particles have dissolved. Keep the

small culture flask in its original position and pour it after cooling to 60°C.

2.2 Inoculation of agarose gel plates

Use tray perfusion gel

1. Keep the provided electrophoresis tank level.

2. Place the plate on the gel stage.

3. Slide the gel plate into the corresponding slot on the gel platform. Ensure that the plate is evenly positioned in the slot and that all edges of the plate are in uniform contact with the tray. The weight of the plate can create a tight seal to prevent any leakage during the gel preparation process.

Note: If a leak occurs when pouring the gel onto the tray on the stage, place the pouring spout in the refrigerator for 2-3 minutes. When preparing to pour the gel, insert the pouring spout into the slot. The cooled spout will prevent the gel solution from leaking out of the tray and into the chamber.

4. Place the comb in the corresponding slot on the tray so that the sample well is close to the cathode (black). During electrophoresis, the DNA sample will move toward the anode (red).

5. Prepare the required concentration and amount of agarose in 1X electrophoresis buffer (see Section 2.1, DNA gel preparation). When the agarose solution has cooled to 50-60°C, pour the melted agarose between the combs of the gel well.

Warning! Hot agarose (>60°C) may cause deformation or cracks in the plate and shorten the service life of the plate. Deformation may also lead to uneven depth of sample holes.

6. Let the gel set at room temperature for 20 to 40 minutes.

7. Carefully remove the comb from the solidified gel. Remove the gel plate.

8. Immerse the gel in 1x electrophoresis buffer solution at a depth of 2-6 mm (see Section 3, Gel and Electrophoresis Reagent Preparation). As the voltage increases, increase the coverage depth (use more buffer solution) to prevent pH-value and thermal effects.

2.3 Electrophoresis

The DL-MINI04 electrophoresis tank supports both manual gel preparation and pre-prepared gel solutions. The system features a gel preparation rack with glass plates equipped with permanent adhesive gel spacer plates, simplifying manual casting processes while eliminating leakage risks. The tank can accommodate 1 to 4 gel layers and is compatible with MiniLab's electrode modules for tank-based electrophoretic transfer and two-dimensional electrophoresis applications.

After the agarose gel has solidified, you can proceed with sample loading and electrophoresis. The agarose gel can be used in various electrophoresis buffers. For nucleic acid agarose gel electrophoresis, Tris-acetic acid-EDTA (TAE) buffer or Tris-boric acid-EDTA is typically employed

(TBE) buffer. Although TAE buffer can make the linear DNA electrophoresis migration speed faster and the resolution of supercoiled DNA higher, TBE buffer has a stronger buffering capacity in the electrophoresis operation with longer time or higher voltage.

1. Prepare the samples to be loaded onto the gel. The loading volume depends on the type of comb used (the thickness and length of the holes) and the thickness of the gel.

2. After the loading volume is determined, add standard nucleic acid loading dye to a final concentration of 1X to increase the density of the sample for easy loading into the sample well (the preparation of the loading dye is described in Part 3 "Preparation of Gel and Electrophoresis Reagents").

3. Load the sample into the well using a standard pipette.

Note: Sample holes are often difficult to observe. Black paper or tape can be placed under the base or tray where the comb is placed and the hole is formed to improve visibility.

4. Carefully cover with the safety cover. Align the red and black banana plugs with the red and black banana plugs on the base.

5. Power requirements will vary depending on gel thickness, length, agarose and its concentration, and the type of electrophoresis buffer used. For relative sample migration rates of different DL-Sub systems and DNA size migration when using loading dyes, please refer to Tables 6 and 7.

Note: Most standard DNA and RNA agarose gel electrophoresis does not require buffer circulation. If buffer circulation is required, simply turn off the power supply, remove the safety lid, and mix the electrophoresis buffer as needed. After mixing the buffer, reseal the safety lid and continue the electrophoresis.

Table 6. Relative migration rates of samples*

Bromophenol blue cell type	Voltage	Mobility
15×15cmgel	75V	3.0cm/hr
15×10cmgel	75V	4.5cm/hr
7×10cmgel	75V	4.5cm/hr

* These sample migration rates were measured on 0.5 cm thick 1.0% agarose gels. Migration rates will vary depending on the voltage, current and type of agarose or buffer used.

Table 7. DNA size migration in the presence of additive dye

Gelatin concentration,%	Xylene cyanol	Bromophenol blue
0.5-1.5	4-5 kb	400-500 bp
2.0-3.0	750 bp	100 bp
> 3.0	125 bp	25 bp

* Screen agarose, such as certified PCR agarose.

** Screen agarose, such as certified low-range superagarose.

2.4 Nucleic acid staining and observation

The gel can be removed from the DL-Sub base or gel tray for nucleic acid staining. The gel can also be left on the gel tray for staining.

Bromoethidium (EtBr) staining step

1. Place the gel in an appropriate amount of 0.5 μg/mL ethidium bromide stain and stain for 15 to 30 minutes. Cover the gel completely with sufficient stain.

Note: Ethidium bromide (EtBr) is suspected to be carcinogenic and should be handled with extreme care. Gloves, safety glasses and lab coats must be worn. Proper handling of used ethidium bromide solutions and gels (see ethidium bromide Material Safety Data Sheet [MSDS] for proper handling).

2. Decolorize the gel in the same volume of dH2O used for staining for 10-30 minutes.

Note: Ethidium bromide (EtBr) can be removed from DNA by prolonged decolorization, which will lead to reduced detection sensitivity. However, insufficient decolorization will produce higher background fluorescence.

3. Briefly rinse the gel with dH2O to remove any residual staining solution.

4. Place the gel on a UV transilluminator for nucleic acid visualization and analysis. The DNA-ethidium bromide (EB) complex can be irradiated with ultraviolet light at 254 nm, 302 nm, or 366 nm. Higher wavelengths result in reduced sensitivity during irradiation, while below 302 nm, DNA cleavage becomes more pronounced. Table 8 provides UV transmittance percentages through 1/4-inch (0.64 cm) UV-transparent plastic (UVTP).

Note: The nucleic acid in the gel can be observed through the tray. If no tray is used, place household plastic wrap between the UV transmittance and the gel to avoid contamination of the transmittance by nucleic acid or ethidium bromide.

Table 8. UV transmittance percentage through 1/4 inch (0.64 cm) UV transparent plastic

Approximate wavelength, nm	Transmissivity %
254	0
302	80
366	90

2.5 Description of the trace method

Nucleic acids in the gel can be transferred to the membrane using Southern blotting and Northern blotting. This manual does not include the steps of the blotting operation, which are beyond its scope. For information about the blotting technique, please refer to the references.

3. Preparation of gel and electrophoresis reagent

RNA agarose formaldehyde gel

For a 100ml 1% agarose formaldehyde gel, prepare it according to the following methods:

- 62 mL of 1.6% melted agarose
- 20 ml of 5× concentration MOPS electrophoresis buffer (final concentration 1 X)
- 18 mL of 12.3 M/L (37.5%) formaldehyde (final concentration 2.2 M/L)

Note: Formaldehyde solution and formaldehyde vapor are toxic.

When handling solutions or gels containing formaldehyde, use a fume hood. Always wear gloves, safety glasses and lab clothes when using formaldehyde. For safety information, see the Material Safety Data Sheet for formaldehyde.

Nucleic acid electrophoresis buffer (see references)

DNA agarose gel electrophoresis typically uses Tris-acetic acid-ethylenediaminetetraacetic acid (TAE) or Tris-borate-ethylenediaminetetraacetic acid (TBE). The TAE buffer enhances the electrophoretic migration speed of linear DNA and improves resolution for supercoiled DNA

TBE buffer exhibits enhanced buffering capacity during prolonged or high-voltage electrophoresis. RNA formaldehyde gels require MOPS [3-(N-morpholinyl)prosylate] electrophoresis buffer.

1× Tris-acetic acid-EDTA (TAE): 40 mM Tris (pH 7.6),20 mM acetic acid and 1 mM EDTA. For a 50x concentrated stock solution (1 L), dissolve the following substances in 600 mL distilled water:

- 242 g Tris base (FW = 121)
- 57.1 ml glacial acetic acid
- 100 ml 0.5M EDTA , pH 8.0

Vigorously shake with distilled water to make up to a final volume of 1 L.

1x tri(hydroxymethyl)aminomethane-boronic acid-ethylenediaminetetraacetic acid (TBE): 89mM tri(hydroxymethyl)aminomethane (pH 7.6),89mL boric acid, 2mL ethylenediaminetetraacetic acid

For the 10x concentrate (1 L), dissolve the following substance in 600 mL distilled water:

- 108 g (FW=121)
- 55g boric acid (FW = 61.8)
- 40 ml 0.5M EDTA , pH 8.0

Vigorously shake with distilled water to make up to a final volume of 1 L.

1× MOPS buffer (RNA gel): 0.02M MOPS (pH 7.0),8mM sodium acetate, 1 mM EDTA 5× concentrate (1 L) dissolved in 600 mL of diethyl pyrocarbonate (DEPC)-treated distilled water:

- 20.6 g 3-(N-morpholinyl)prosulfonic acid
- 13.3 mL 3M sodium acetate (treated with diethyl pyrocarbonate), pH 7.4
- 10 ml 0.5M ethylenediaminetetraacetic acid (tetrabenzoyl pyroate treated), pH value 8.0

Vapidated water treated with diethyl pyrocarbonate (DEPC) was made up to a final volume of 1 L.

Note: DEPC is a suspected carcinogen. Always wear gloves, safety glasses and lab coat. Handle DEPC-containing solutions with care. For additional information, see the Material Safety Data Sheet for diethyl pyrocarbonate.

DNA and RNA staining dyes (see references)

Prepare each RNA sample as follows before loading RNA onto the agarose formaldehyde gel:

- 6 ul RNA (dissolved in water treated with DEPC)
- 10 µl of MOPS buffer at 5× concentration (final concentration 1.67 times)
- 9 ul 12.3 M formaldehyde (final concentration 3.7 M)
- 25 µl formamide (final concentration 50% V/V)

Note: Formamide is a teratogen. Always wear gloves, safety glasses and lab clothes. Handle formamide with care. For more information, see the Material Safety Data Sheet for formamide.

Bromoethidium solution

Add 10mg ethidium bromide to 1ml distilled water.

4. Maintenance and maintenance

4.1 Clean DL-Sub system components

1. All DL-Sub parts should be cleaned in warm water with a mild soap or detergent solution.

Note: When cleaning, be careful not to hook or break the electrode wires in the base.

2. Thoroughly rinse all parts with warm water (distilled water if possible) and then air dry.

4.2 Compatible cleaning agents

Chemically compatible cleaning agents must be used to ensure long service life of components. These cleaning agents include:

-A solution of soap or mild detergent

▪ organic solvent ：

– hexane

– fatty hydrocarbon

Do not soak plastic parts in detergent for more than 30 minutes. Usually just rinse them with detergent.

Note: Do not use the following chemicals to clean DL-Sub parts. Contact with these chemicals may cause plastic parts to crack, silver streaks, etching or deformation.

▪ chlorhydrocarbons

– carbon tetrachloride

– chloroform

▪ aromatic hydrocarbon

– benzene

– phenol

– methylbenzene

– methyl ethyl ketone

– acetone

▪ alcohols

– carbinol

– alcohol

– isopropanol

Do not use abrasive or strongly alkaline cleaning agents for DL-Sub components.

Do not expose subcell components to temperatures (>60°C). Do not use autoclave or dry heat to sterilize subcell components.

4.3 Maintenance time

For instructions on maintaining your DL-sub system, see Table 9.

Table 9. System maintenance

Project	Question	Frequency	Operate
All parts	Dry salt, agarose, oil and dirt	concrete purpose	Clean the parts as described in Section 4.1
Electrical wires	Breakage or wear	Use it every time	restringing
pallet	Chip or crack	concrete purpose	Change the tray
wire electrode	circuit breaker	concrete purpose	See section 4.4
Wire connections (banana plugs and connectors)	become less crowded	weekly	Replace the banana plug or socket

4.4 RNase decontamination

Before performing RNA gel electrophoresis using any DL-Sub system, the components should be cleaned with a mild detergent and treated with 3% hydrogen peroxide (H2O2) for 10 minutes. The components must then be rinsed with 0.1% diethyl pyrocarbonate (DEPC)-treated distilled water to remove RNA enzymes. For additional recommendations regarding the use of DEPC in RNA enzyme decontamination, please refer to the reference materials.

Note: DEPC is a suspected carcinogen. Always wear gloves, safety glasses and lab clothes. Handle DEPC-containing solutions with care. For more information, see the Material Safety Data Sheet for diethyl pyrocarbonate.

Do not attempt to remove ribonuclease contamination from Sub-Cell GT components using the extremely dry heat method.

5. Troubleshooting

Table 10. Troubleshooting

Question	Possible causes	Rx
The stripe is blurred or trailing	The gel is not uniform and the voltage is too high	Check the level of glue preparation and reduce the voltage to 5V/cm
The addition port is ruptured	Improper way of plucking	Gently pull one side of the comb, avoiding vertical force
Buffer leak	The tray is not sealed or the tape is not fastened	Re-seal both ends of the tray
Electrode corrosion	The buffer was not replaced in time	Change the buffer regularly and clean the electrode
Cross-contamination of high-throughput samples	The sampling interval is too dense	Use a standard sampling gun to avoid bubbles
The gel cracked	Voltage gradients are too high, especially when using agarose with a lower melting point or a gel of lower strength.	Lower the voltage and run the gel at a lower temperature

6. References

1. Sambrook J et. al. (1989}. Molecular Cloning, A Laboratory Manual, Second Edition(Col d Spring Harbor Laboratory Press,

2. Current Protocols in Molecular Biology, Greene Publishing Associates andWiley-nterscience, 1989.

remedial reading

3. Kopchick, 丿. j., Cullen, B. R. and Stacey, D. , Anal Biochevn.. 115, 419 (1981).

4. Southemn, E., Methods in EnzyoL, 68, 152 (1979).

5. The Bio-Rad Silver Stain - Bullelin 1089, Bio-Rad Laboratories, Hercules, CA.

6. Bittner, M.. Kuplerer, P, and Morris, C .F.. Anal Biochern, 102, 459 (1980).

7. Bio-Rad Trans-Blot Cell Operation Instruclions, Bulletn 1082, Bio-Rad laboralories,Hercules, CA.

8. Winberg, G. and Hammarskjold, M. .. Nucleic Acids Res.. 8. 253 (1980).

9. ytatekadze, 1. v. Axelrod, V. D. Gorbulev, v. G, Belzhelarskaya, $. N. and Vartikyan, P. ...Anal. Biochem..100.129 (1979}.

10. Dretzen, G, Belerd, M., Sassone-Corsi, P, and Chambon, P, Anal. Biocharn, 112, 295 (1981)

7. 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.