Lonza

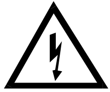
Lonza Rockland, Inc.
www.lonza.com
biotechserv@lonza.com
Tech Service: 800-521-0390
Customer Service: 800-341-1574
Document # 18928-0305-01
Rockland, ME 04841 USA

PAGEr™ Minigel Chamber

IMPORTANT USER INFORMATION

This Instruction Manual will explain how to use this product safely and effectively. Please read and carefully follow the instruction manual in its entirety.

The triangle/exclamation mark symbol alerts the user of the product to important operational, maintenance, and/or warranty requirements.



The triangle/lightning bolt symbol alerts the user of the product to potentially hazardous electrical exposure.

Failure to adhere to the instructions could result in personal and/or laboratory hazards, as well as invalidate any warranty. Always turn off the DC power source prior to disconnecting power cords from the product. Disconnect power cords from the power source first and then from the product. For maximum safety, always operate this system in an isolated, low traffic area, not accessible to unauthorized personnel. Never operate damaged or leaking equipment.

WARRANTY AND LIABILITY

This product was produced utilizing the highest practical standards of materials, workmanship, and design. Lonza Rockland, Inc. warrants that the product has been tested and will meet or exceed published specifications. This warranty is valid only if the product has been operated and maintained according to the instructions provided.

Lonza Rockland, Inc., warrants this product to be free from defects in materials and workmanship under normal service for one year from date of shipment. If the product proves defective during this period, Lonza Rockland, Inc., will repair or replace it at our option, free of charge, if returned to us postage prepaid. This warranty does not cover: damage in transit, damage caused by carelessness, misuse or neglect, normal wear through frequent use, damage caused by solvent corrosion, damage caused by improper handling or user alteration, nor unsatisfactory performance as a result of conditions beyond our control. Lonza Rockland, Inc., shall in no event be liable for incidental nor consequential damages, including without limitation, lost profits, loss of income, loss of business opportunities, loss of use and other related damages, however caused, nor any damage arising from the incorrect use of the product.

SECTION 1
General Information

1.1 Introduction

The PAGEr Minigel Chamber is designed for optimal performance of PAGEr® Precast Gels (Lonza).
The unit is also compatible with many standard precast minigels. The simple lock-in-place core design assures a tight, flat fit and eliminates the risk of buffer leaks. There is no need to remove the core from the reservoir, simply insert the gels, close the clamps, fill with buffer and run. The chamber can run one or two gels and accommodates a tank blotting module.

Table 1: Components of the PAGEr Minigel Chamber

Cat No	Description
59905	PAGEr Minigel Chamber
59906	PAGEr™ Blot Module
59907	PAGEr Minigel Chamber and Blot Module Kit (Includes chamber, blotting cassettes and sponge pads)

1.2 Safety

Power to the PAGEr Minigel Chamber is to be supplied by an external DC voltage power supply that must be ground isolated so that the DC voltage output floats with respect to ground. For any power supply used, the maximum specified operating parameters for the units are:

Maximum Limits
250 VDC
30 watts power
150 mA current
60ºC ambient temperature

Current to the unit, provided from the external power supply, must enter the unit through the lid assembly, providing a safety interlock to the user. Current to the unit is broken when the lid is removed.
Do not attempt to use the unit without the safety lid, and always turn the power supply off before removing the lid, or when working with the unit in any way. Follow safety precautions specified by the power supply manufacturer.

Important: After each use, rinse all parts with de-ionized water.

SECTION 2
Description of Parts
2.1 Unpacking and Components
Please verify that your unit comes complete with the following components:
- Lower reservoir
- Safety cover with attached DC power leads
- Core
- 1 blank plate for running single gels (not shown)
- 2 adaptors for running 9 cm gels (not shown)

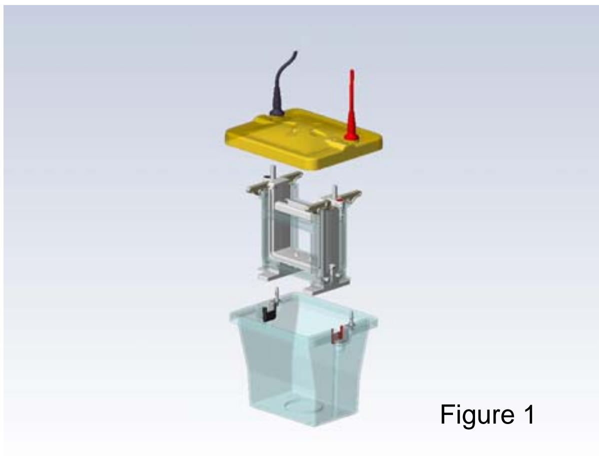


Figure 1

Section 3
Instructions for Electrophoresis

3.1 Preparing the Electrophoresis Unit

1. Place unit in authorized work area. Remove safety cover from the assembled unit by simultaneously pressing down on white push pins with your thumbs, while lifting up on yellow safety cover with your fingers. **Do not remove safety cover by pulling up on leads!**

Figure 2

2. Remove white core from lower reservoir by grasping core with one hand and lifting directly up.
NOTE: If desired, gels may be inserted without removing the core from the reservoir.

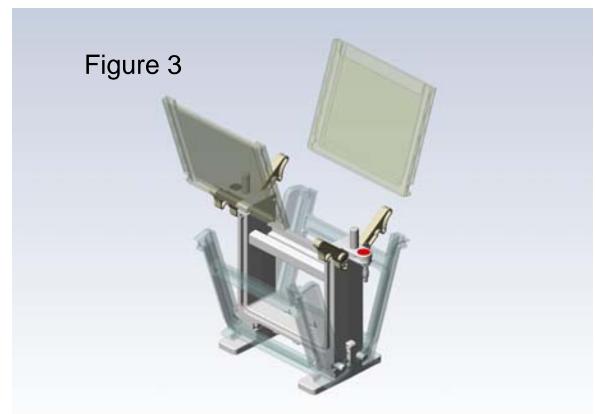


Figure 3

3. Open doors on the core assembly by pulling up on the white latches.

4. Slide pre-cast gel cassette into the core assembly with the notched plate facing in towards the upper buffer reservoir as shown in figure 3.

5. If running one gel, slide the blank plate into the side without the gel. If running a 9 cm x 10 cm cassette, insert the 9 cm gel adapter, as shown in figure 4.

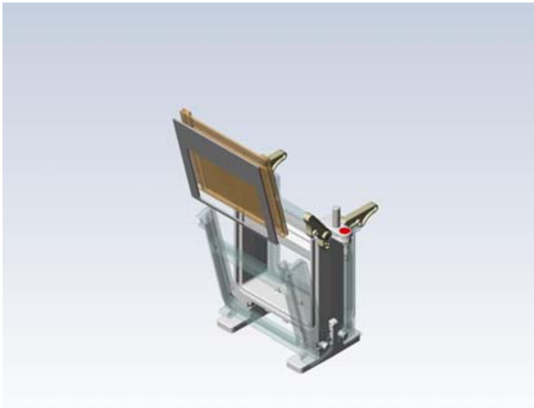


Figure 4

6. Close doors and relatch by pressing down on the white latches so that the assembly looks like that shown in figure 5.

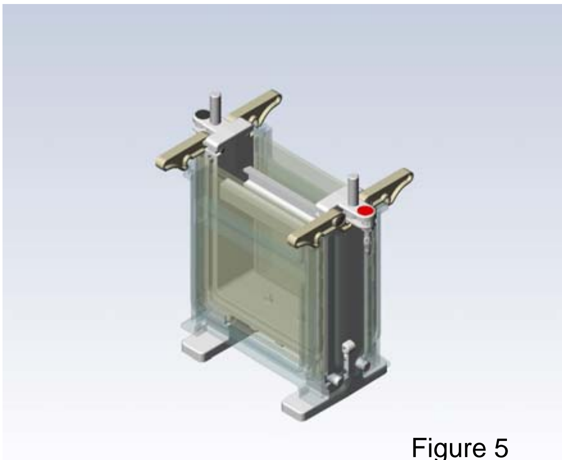


Figure 5

7. Place stirring bar, if desired, into bottom of reservoir in stirring corral.

3.2 Running the Gel

1. Place core assembly into lower reservoir. The anode (red) and cathode (black) electrodes are color-coded on both the core/cassette assembly and lower reservoir. Ensure the red dot on the cassette assembly is on the same side as the red receptacle on the lower reservoir. Fill core upper reservoir with freshly prepared buffer (~ 190 ml).
NOTE: **<u>If any buffer is spilled into banana jack receptacle in lower reservoir, dry completely using compressed air!</u>** **<u>Failure to do this will result in accelerated banana jack corrosion.</u>**

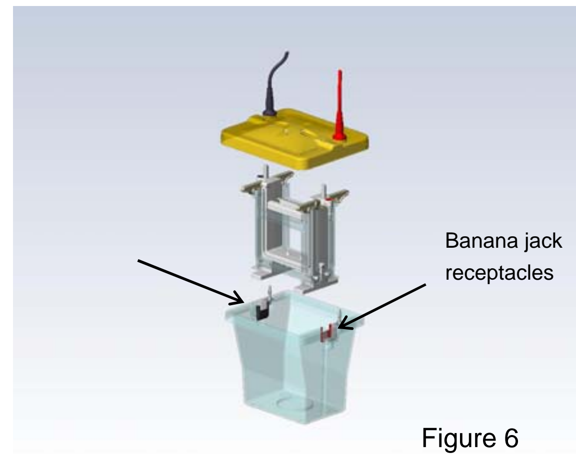


Figure 6

2. See section 5.3 for recommended buffers. Pour only enough freshly prepared buffer into lower chamber so that the final buffer level is just below bottom of sample wells. Using a pipette or syringe, thoroughly flush out the wells.
3. Load samples. If outer lanes do not contain sample, it is recommended that you run standards and/or fill outer lanes with loading buffer to reduce smiling and wrap-around effects.
4. Attach safety cover and turn on magnetic stirrer (if desired).

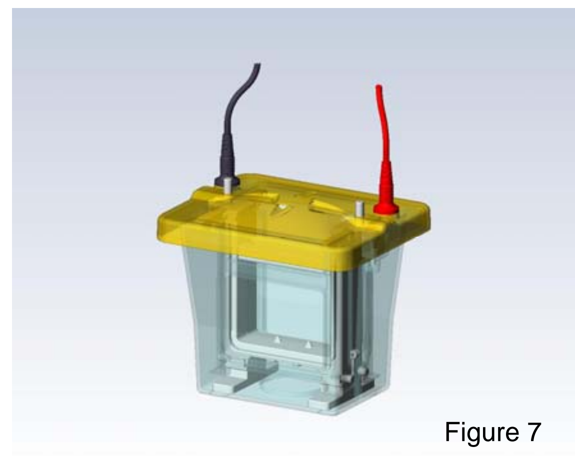


Figure 7

5. Connect the leads to the power supply, matching the color-coded red-to-red and black-to-black. **See Section 5.1 for recommended power conditions.** Begin separation by electrophoresis. Figure 7 shows the closed unit ready for electrophoresis.

3.3 Removing the Gel

1. Turn the power supply off and disconnect the leads from the power supply. Remove the safety cover from the unit, by simultaneously pressing down on the white push pins with your thumbs, while lifting up on the yellow safety cover with your fingers. See Figure 2. **Do not remove safety cover by pulling up on leads!**

2. Pull up on gel door latches, and open gel door. Remove gel cassette.

3. Fix and stain gel according to your preferred method.

Section 4
INSTRUCTIONS FOR WESTERN BLOTTING

4.1 Preparing the Unit for Blotting

1. Remove safety cover from the assembled unit by simultaneously pressing down on white push pins with thumbs while lifting up on yellow safety cover with your fingers. See figure 2. **Do not remove safety cover by pulling up on leads!** Remove white core from lower reservoir by grasping core with one hand and lifting directly up. Open doors on the core assembly by pulling up on the white latches.
2. Open blotting cassette (as shown in figure 8) and lay it flat on the bench.

Figure 8

3. **Western Blotting Instructions.**

   a. Soak gel in 1X Towbin (1X tris-glycine buffer/ 20% Methanol) buffer for 10 minutes.
   b. Cut a piece of PVDF or nitrocellulose membrane to fit the gel. **Do Not Touch Membrane with Bare Hands. Wear gloves.**
   c. Soak membrane in 50% methanol for 30 seconds.
   d. Soak membrane in water for 5 minutes.
   e. Soak membrane 5 minutes in 1X Towbin buffer until it no longer floats. **Do Not Allow Membrane to Dry.**
   f. Cut 1 piece of filter paper 1 mm thick or less to fit the gel.
   g. Soak sponge in 1X Towbin Buffer.

4. Assemble blotting stack. With blotting cassette wide open assemble components on black side in the following order, as shown in Figure 9: foam pad, gel*, buffer saturated transfer membrane, then buffer saturated blotting paper. Smooth with gloved finger or roll with glass rod to be sure no bubbles exist between the gel and the transfer membrane.

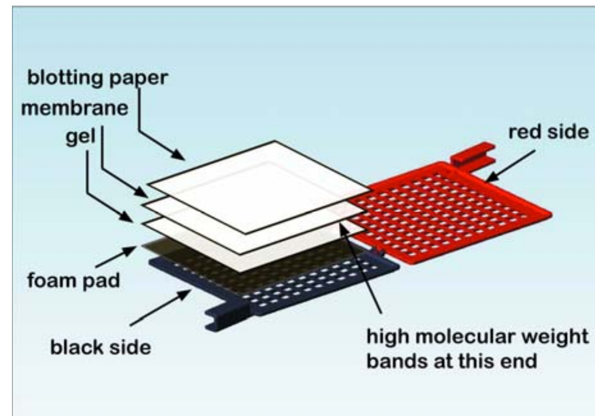


Figure 9

***NOTE: to prepare gel for blotting, trim off wells and any excess gel at the bottom, and invert 180º so that the large molecular weight proteins are at the bottom of the cassette. This puts the large proteins in contact with a stronger field strength and allows the blotting transfer to take place more efficiently.**

5. Insert blotting cassettes into core making sure that red side faces outward, as shown in figure 10.

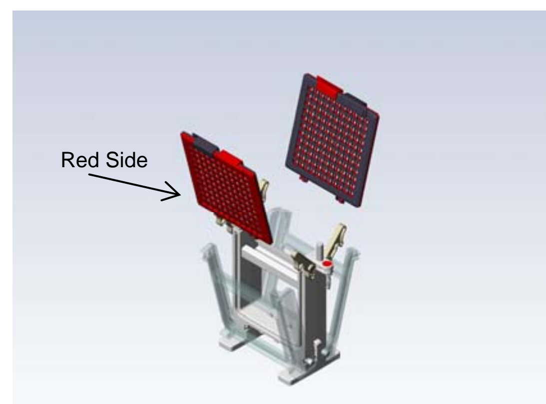


Figure 10

6. Close doors and re-latch by pressing down on the white latches. If running one blot, slide blank adapter plate into the side without the blotting cassette.

4.2 Electro-Blotting Procedure

1. Place stirring bar in bottom of reservoir in stirring corral. Place core/blotting cassette assembly into lower reservoir. The anode (red) and cathode (black) electrodes are color-coded on both the core/cassette assembly and lower reservoir. Ensure the red dot on the cassette assembly is on the same side as the red receptacle on the lower reservoir.
2. Pour 1 liter of freshly prepared, chilled (4º) Towbin buffer into lower buffer reservoir. Buffer will percolate into central core.
3. Attach safety cover, as shown in figure 11.

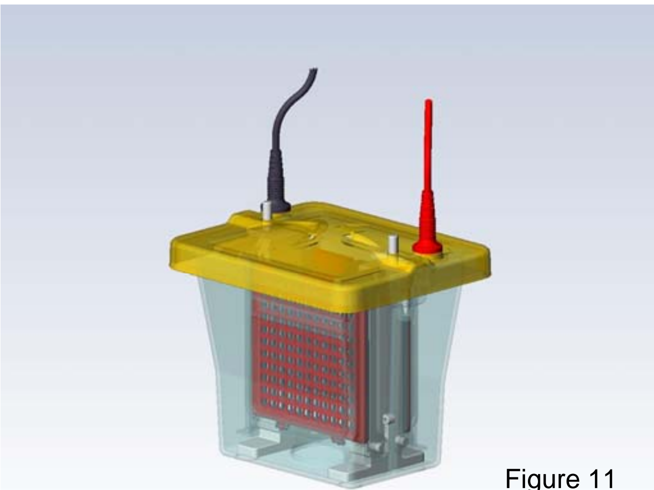


Figure 11

4. Connect the leads to the power supply, matching the color-coded red to red and black to black. **See Section 5.2 for recommended power conditions.** Begin transfer by electrophoresis.

4.3 Removing the Blot

1. Turn the power supply off and disconnect the leads from the power supply. Remove the safety cover from the unit, by simultaneously pressing down on the white push pins with your thumbs, while lifting up on the yellow safety cover with your fingers. See Figure 2. **Do not remove safety cover by pulling up on leads!**
2. Blotting cassettes can be removed by leaving the core in place and opening the top latches of the core, opening the doors and lifting the cassettes out. Unlatch the blotting cassettes and remove blot from blotting sandwich.

SECTION 5
Running Conditions

5.1 Recommended Power for Slab Gels:

Precise electrophoresis conditions will vary according to the number and type of gels used, buffer conditions employed, power input, and the general goal of the experiment. Refer to section 5.4 for in depth discussions on practical and theoretical approaches to protein gel electrophoresis.

Using standard SDS-PAGE buffer systems (see section 5.3). For **two** 1.0mm thick gels at room temperature use the following conditions at constant voltage:

200 VDC for ~60 minutes, or if faster runs are desired, 250 VDC for 30 minutes.

As the thickness of gel increases, the mA's increase proportionally.
At constant voltage 200-250 VDC, the proteins will migrate at a constant rate during electrophoresis with adequate heating appropriate for denaturing gels. Increasing the voltage/mA (for each single gel thickness and percentage) will speed mobility but increase the risk of overheating.

5.2 Recommended Power for Electro-Blotting:

Using standard SDS-PAGE electro-blotting buffer systems (see section 5.3) use the following conditions:
100 VDC @ 90 minutes.

5.3 Recommended Buffers

Protein Denaturing Buffer:	**Protein Electro-Blotting Buffer:**
TG-SDS (1X):	**TG-SDS Towbin (1X) 20% MeOH:**
0.025M Tris base	0.025M Tris base
0.192M Glycine	0.192M Glycine
0.1% (w/v) SDS	0.05-0.1% (w/v) SDS
pH 8.3	20% (v/v) Methanol

For Best Results use AccuGENE® Electrophoresis Buffers (see ordering info).

5.4 References

1. Hames, B.D. (ed.) (1998). *Gel Electrophoresis of Proteins. A Practical Approach.* 3rd edn. Oxford University Press, Oxford. Ch. **1**,**3**.
2. Sambrook, J., Fritsch, Russell, D. (2001). *Molecular Cloning. A Laboratory Manual.* 3rd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York. A8.40-A8.55
3. Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A., Struhl, K. (ed) (1993). *Current Protocols in Molecular Biology*. Vol.

2, Greene Publishing Associates, Inc. and John Wiley & Sons, Inc., Ch. **10**.

SECTION 6
Maintenance of Equipment

6.1 Care and Handling

The plastic components of the PAGEr™ Minigel Chamber are fabricated from polycarbonate. Electrodes and connectors are made from pure platinum, stainless steel, and nickel plated brass. As with any laboratory instrument, adequate care ensures consistent and reliable performance.

After each use, rinse all parts with de-ionized water. Wipe dry with a soft cloth or paper towel, or allow to air dry. Whenever necessary, all components may be washed gently with water and a non-abrasive detergent, and rinsed and dried as above. *Never* use abrasive cleaners, glass cleaning sprays or scouring pads to clean the components, as these will damage the unit and components.

Additional precautions:

- Do not autoclave or dry-heat sterilize the apparatus or components.
- Do not expose the apparatus or components to phenol, acetone, benzene, halogenated hydrocarbon solvents or undiluted alcohols.
- Avoid prolonged exposure of the apparatus or components to UV light.
- Do NOT treat with diethylpyrocarbonate (DEPC)-treated water for extended periods at 37ºC. A brief rinse with DEPC-water is sufficient after a thorough wash, followed by a quick rinse in 70% ethanol.

6.2 Maintenance

The following inspection and maintenance procedures will help maintain the safety and reliable performance of the PAGEr Minigel Chamber. Replacement parts can be requested by calling 1-800-638-8174.

- Banana plugs and power cords should be inspected regularly. If the banana plugs become loose or do not feel friction tight replace the plugs or power cords.
- Should power cord assemblies (connectors, wire or shrouds) show any signs of wear or damage (e.g. cracks, nicks, abrasions, or melted insulation), replace them immediately.
- The platinum wire is secured to the banana jack by compression between a stainless washer and the jack nut. The nut/washer interface should be tight and free of corrosion.

Lonza

Ordering Information

Cat No	Description
59905	PAGEr Minigel Chamber
59906	PAGEr™ Blot Module
59907	PAGEr Minigel Chamber and Blot Module Kit (Includes chamber, blotting cassette and sponge pads)
50879	AccuGENE® 10X Tris-Glycine Buffer 1L
50881	AccuGENE 10X Tris-Glycine Buffer 4L
50880	AccuGENE 10X Tris-Glycine SDS Buffer 1L
50882	AccuGENE 10X Tris-Glycine SDS Buffer 4L

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