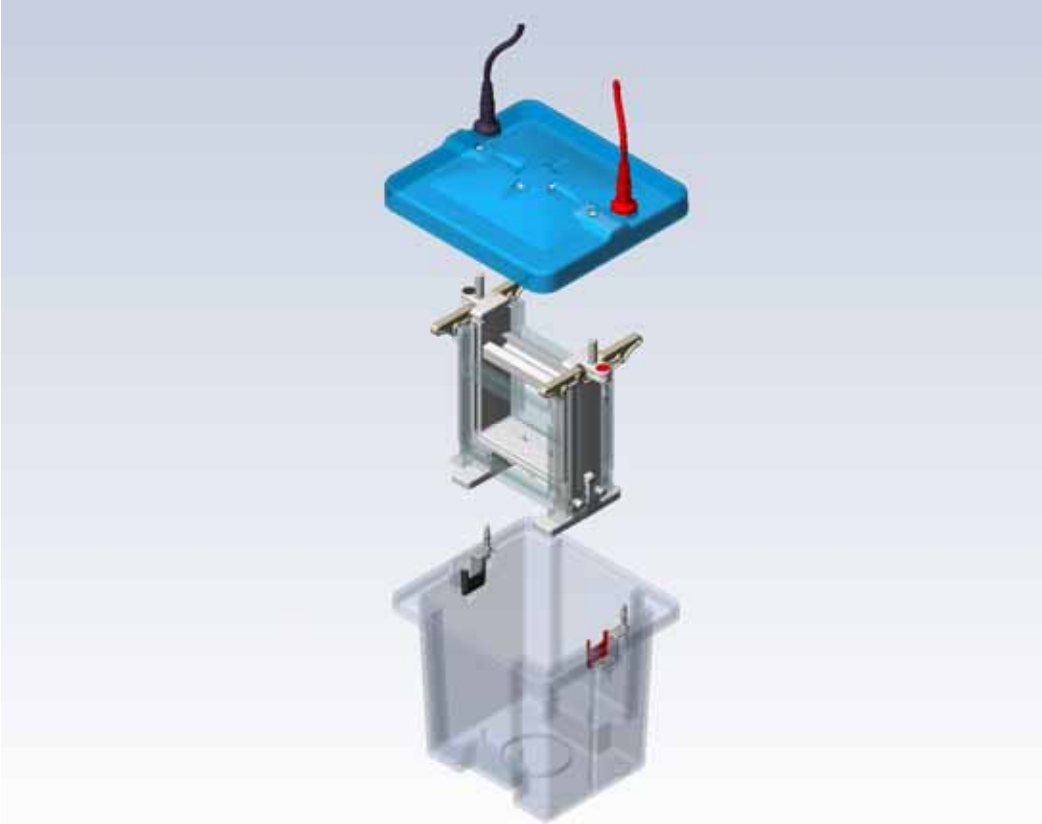
Nacalai USA
Innovations for Life Sciences.

CosmoPage Dual Run & Blot System



I N S T R U C T I O N M A N U A L

Nacalai USA, Inc.
6640 Lusk Blvd. Suite A200
San Diego, California 92121 USA
TEL (858) 404-0403 ▪ Fax (858) 404-0408
Email: info@nacalaiusa.com ▪ www.nacalaiusa.com

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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/lighting 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. Nacalai USA, 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.

Nacalai USA, 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, Nacalai USA, 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. **Proper use of the unit requires thorough rinsing of the components, including immersing the buffer core in water after use as further described in section 5.** Nacalai USA, 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

Nacalai USA, Inc. offers the CosmoPAGE Dual Cell & Blot System for performing vertical electrophoresis separations and electroblotting. The unit provides the capability of running and transferring two gels simultaneously under identical conditions. The unique voltage gradient during blotting (patent pending) provides a field-strength gradient inversely proportional to the distance the protein or polynucleotide has run in the gel. Units include a white plastic buffer dam when running and blotting one gel at a time.

1.2 Specifications

Materials:	
Reservoir chamber, safety cover	Acrylic/Polycarbonate
Electrophoresis Core Assembly	Polycarbonate, stainless steel
Electrodes	Platinum wire .012" diameter
Power cords	FR Urethane rated 7500VDC, 200mA, 65 °C
Safety Certification	EN61010-1-1993 (IEC1010-1)
Unit Dimensions	
Width	7 inches (17cm)
Depth	5 inches (13cm)
Height	8 inches (20cm)
Shipping Weight	4 pounds (2 kg)

1.3 Safety

Power to the CosmoPAGE Dual Cell & Blot System 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 unit are:

Maximum Operating Limits

Voltage:	250 VDC
Power	50 watts
Current	300 mA
Ambient temperature	60 °C

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

SECTION 2

Description of Parts

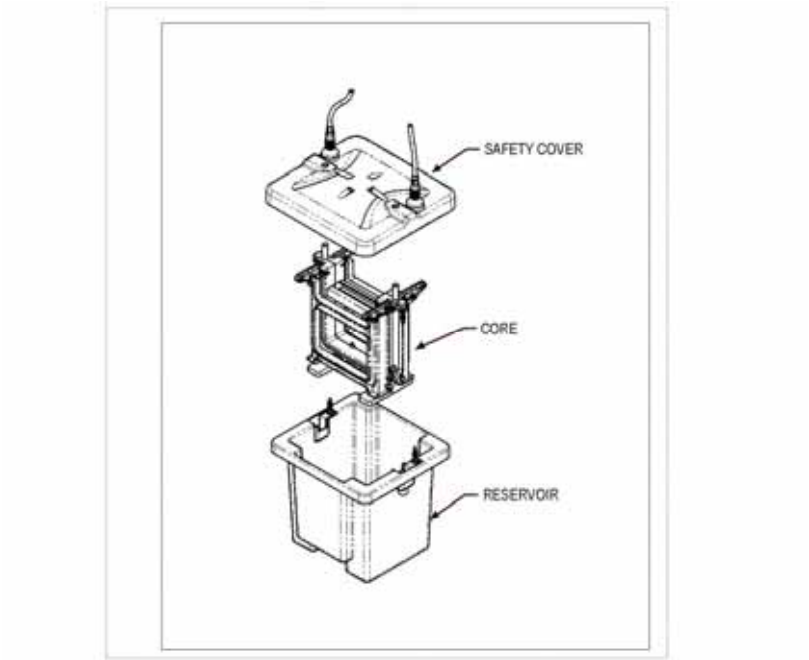
2.1 Unpacking

Please verify that your unit comes complete with the following components:

CosmoPAGE Dual Run & Blot System Package Contents

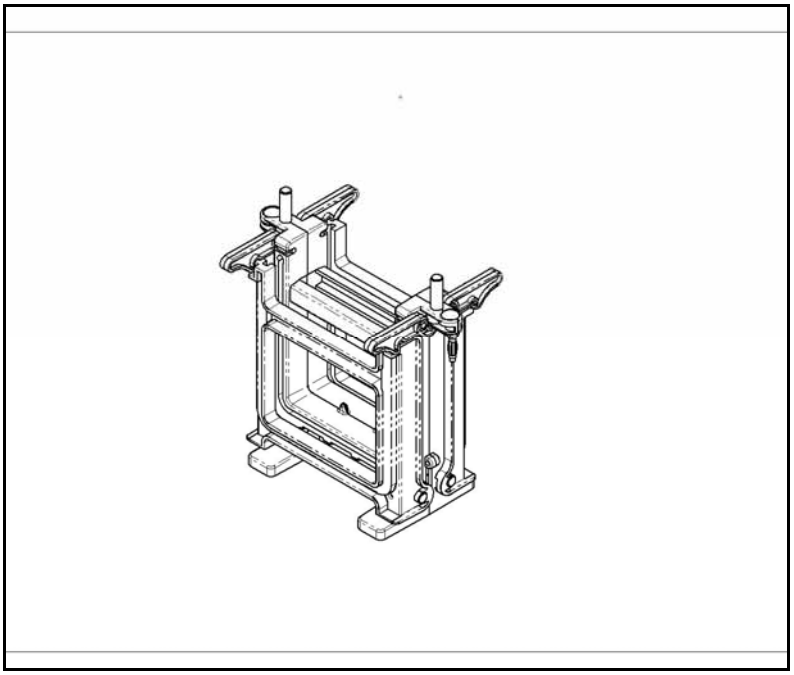
- Lower Reservoir Assembly
- Safety Cover with attached DC power leads
- Electrophoresis Core Module
- Blotter Cassettes, 2 each
- Package of 6 foam pads
- Buffer Dam
- Cooling Blocks, 2 each

"Figure 1: CosmoPAGE Dual Run & Blot System – Exploded View"

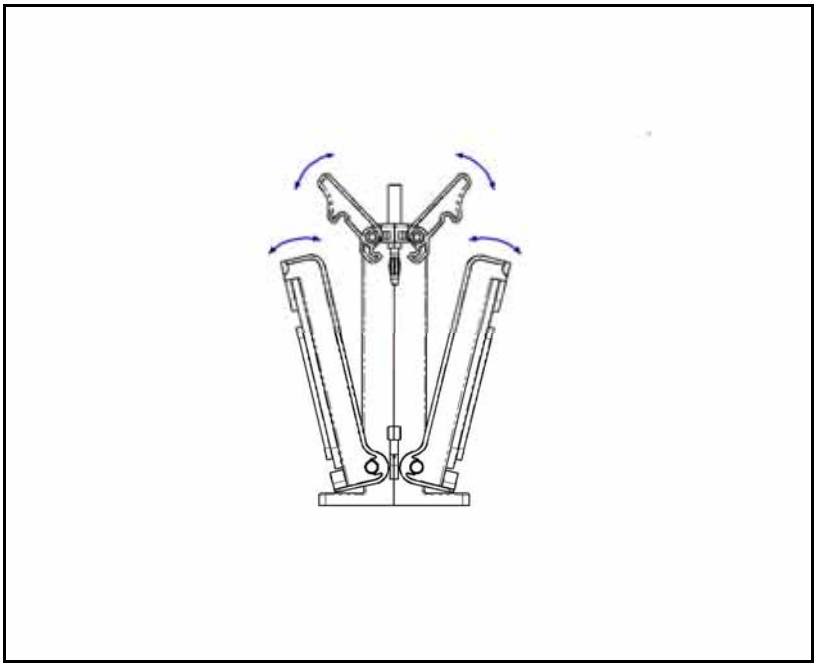


"Figure 2: Electrophoresis Core Module "

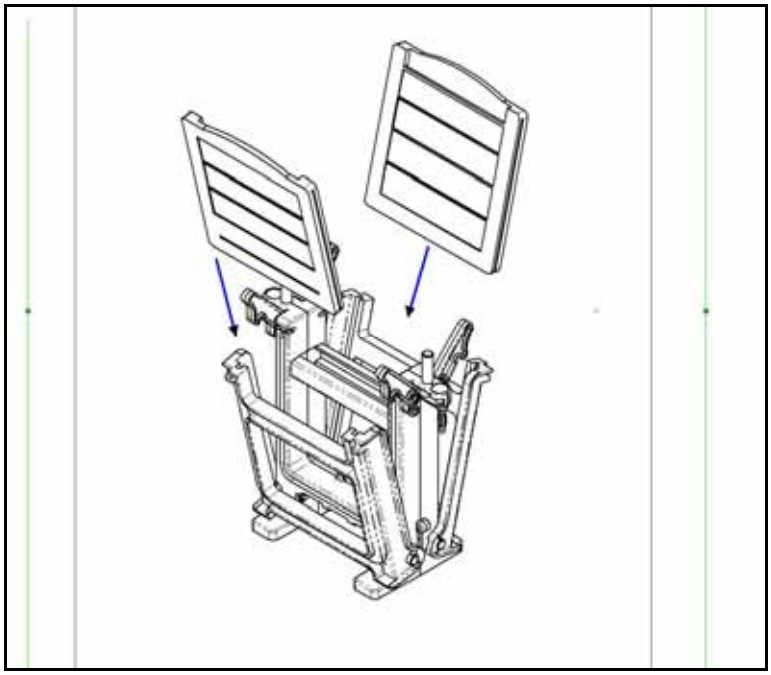
"Figure 2A: Core Module - Closed Position "



"Figure 2B: Core Module - Open Position "



"Figure 2C: Inserting CosmoPAGE Gel Cassettes into the Core Module"



SECTION 3

INSTRUCTIONS FOR RUNNING CosmoPAGE GELS

3.1 Running Buffer Preparation

The CosomoPAGE SDS Gel Run Buffer formulation must be used to run these gels. Prepare 600 ml of 1x running buffer for one run. For reduced samples in standard SDS-PAGE separations, dilute **30ml** CosmoPAGE SDS-Std Run Buffer Reduce (20x) (NU60500 or NU60525) with **570ml** ultrapure water. For non-reduced samples in standard SDS-PAGE separations, use CosmoPAGE SDS-Std. Run Buffer (20x) [NU50500 ot NU50525], diluted the same. For reduced samples in NuPAGE™ MES-like separations, use the CosmoPAGE SDS-Low Range Run Buffer Reduce (20x) (NU13500 or NU13525), diluted the same. The buffer may be reused on the outer (anode) side, but **fresh buffer is always required in the inner (cathode) chamber.** When saving buffer for reuse, be sure to mix the used inner and outer chamber buffers. When reusing buffer, add **10** ml of CosmoPAGE Running Buffer (20x) to **190** ml ultrapure water for the inner chamber.

3.2 Loading Samples onto the Gel

Shortly before loading samples, rinse the wells two times with ultrapure water and fill lanes with fresh Running Buffer or ultrapure water. Load samples by under-laying them in the wells. Use thin pipette tips to load samples near the bottom of the well. Maximum sample capacity is 35 µl for 12-well gels and 17 µl for 17-well CosmoPAGE Gels.

3.3 Running the Gels

1. Open doors on the Core Module by pulling up on the white latches (See Figures 2A & 2B). The gel cassettes are placed in the cell with the shorter plate facing in towards the inner chamber (See Figure 2C). Slide the gel cassette(s) into the Core Module. If running one gel, use the buffer dam to seal the other side. Close doors and re-latch.

2. Place Core Module with the gels into Lower Reservoir Assembly (See Figure 1). The anode and cathode electrode are identified on the Core Module and on the Lower Reservoir Assembly with a red or black circle. Ensure the red dot on the cassette assembly is on the same side as the red dot on the lower reservoir.

3. Fill the inner chamber with **200 ml "fresh"** 1x Running Buffer until it overflows into the outer chamber. If the cell is assembled properly so that there are no leaks, then pour **at least 400 ml** Running Buffer into the outer chamber.

4. Attach the Safety Cover.

5. Connect the leads to the power supply, matching the color code: red to red, and black to black. Run the gel(s) under the following conditions until the blue dye front nears the bottom of the cassettes (40 to 90 minutes, depending on gel percentage).

Gel%	Run Voltage	Starting Current	Ending Current	Run Time
8%, 4-8%, 10%, 4-12%	150VDC	60mA/gel	30mA/gel	45-60 minutes
12%, 4-20%, 16%, 10-20%	200VDC	90mA/gel	40mA/gel	40-70 minutes

3.4 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 placing thumbs on white posts next to red & black connectors, then pushing down while pulling up with fingers under lid**. DO NOT pull on the power cables.**

2. Pull up on gel door latches, and open gel door. Remove gel cassette(s) from the Core Module. Open the cassette using a comb or gel knife, gently lever apart four corners of the cassette, first on one side, then the other. For gel staining, follow the Running Instructions for the appropriate type of Cosmo P A G E Cassette Gel and staining method.

3. Rinse chamber, lid and buffer core **thoroughly** with de-ionized water. **The buffer core must be immersed in water to rinse buffer contaminants of the cavities.** Failure to properly rinse the buffers out of the cell components can lead to chemical attack and will void your warranty.

SECTION 4

INSTRUCTIONS FOR BLOTTING

The CosmoPAGE Dual Cell & Blot System can blot one or two CosmoPAGE Gels. For uniform transfers, using a magnetic stir bar and stirrer facilitates buffer circulation and heat exchange.

4.1 Blotting Setup

Recommended Transfer Buffer and Transfer Conditions for CosmoPAGE Gels

Gel Type	1x Transfer Buffer	Blotting Conditions
Denaturing (Western)	**25mM Tris / 192mM Glycine / 0.1%SDS / Methanol** **Membrane Type** **Nitrocellulose PVDF** **20x TGS Run/Blot Buffer** **(Cat # NU82500) .....100 ml.....100 ml** **Methanol .....200 ml.....100 ml** **Ultra-pure water .....720 ml.....810 ml** **Starting buffer temperature: 20 to 25°C.**	**200VDC Constant for 60 to 90 minutes** **Expected Current: About 215mA for 2 gels, 180mA for 1 gel at ca. 22°C**

4.2 Preparation of Electroblotting Components

1. After running the CosmoPAGE Gel and removal from the cassette, pre-equilibrate the gel with the 1x transfer buffer for 5-10 minutes prior transfer. **Use room temperature buffer for best results.**

2. Equilibrate pre-cut membranes (Nitrocellulose or PVDF) in 1x transfer buffer for 3-5 minutes. Note: PVDF must be wetted in 100% methanol or ethanol prior to equilibration in buffer.

3. Soak pre-cut 1 mm thick filter paper and a foam pad in 1x transfer buffer.

4. Open the Blotting Cassette (see Figure 3A) and submerge it in a shallow dish. Fill with enough buffer to cover the entire cassette. Layer a piece of 1 mm thick filter paper on top of the **black** panel (-).

5. Layer the gel on top of the filter paper. **Apply gel orientation with High Molecular Weight bands** down (near hinges) and close to the blotting cassette connection point (see Figure 3B).

6. Apply blotting membrane. Remove trapped air bubbles at each layer by rolling a pipette over the surface. Note: Some proteins will begin transfer immediately upon contacting the transfer membrane. Moving the membrane across the gel can result in a smeared blot.

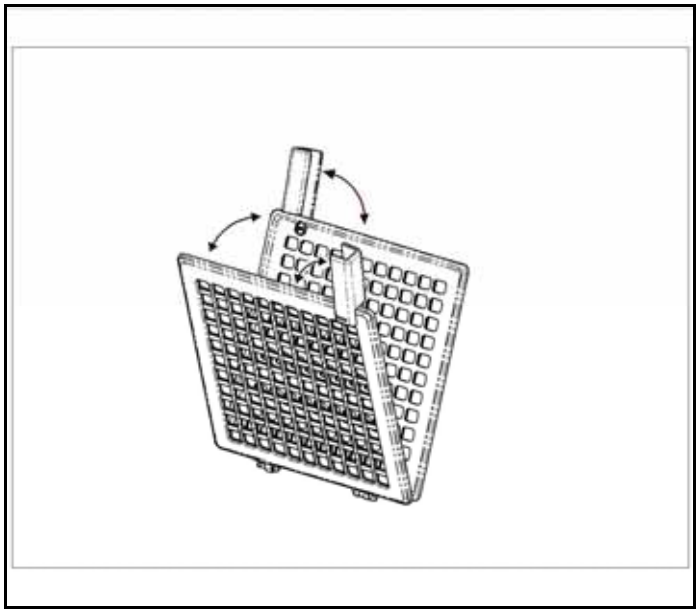
7. Submerge the folter paper and the foam pad. Place the folter paper and the pad on top of the membrane to complete sandwich assembly. Close the blotting cassette (**red** panel [+]).

8. Remove entire assembly from the dish and load into desired position (slot) in buffer chamber with black side(s) facing in towards the Core Module (see Figure 3C).

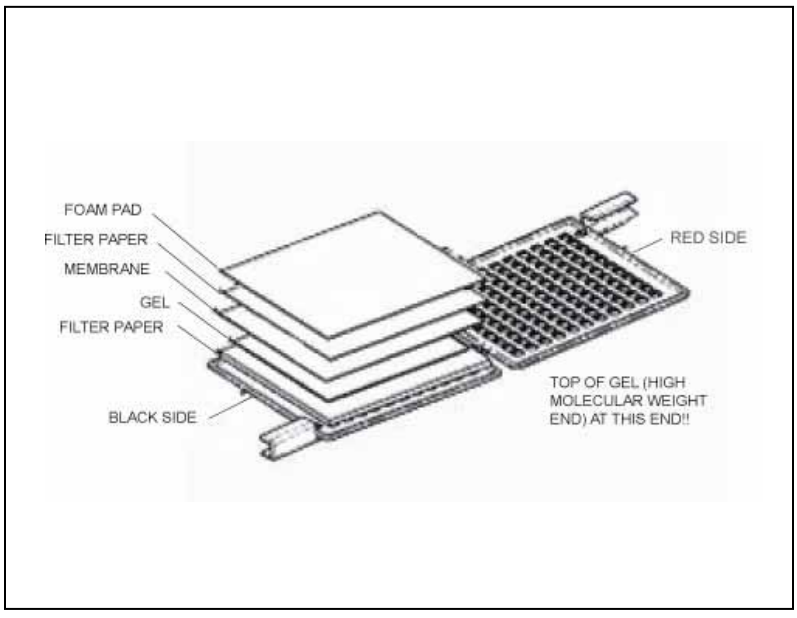
9. When blotting one gel, place the Buffer Dam in the unused position.

"Figure 3: Electrophoresis Blotting Cassette "

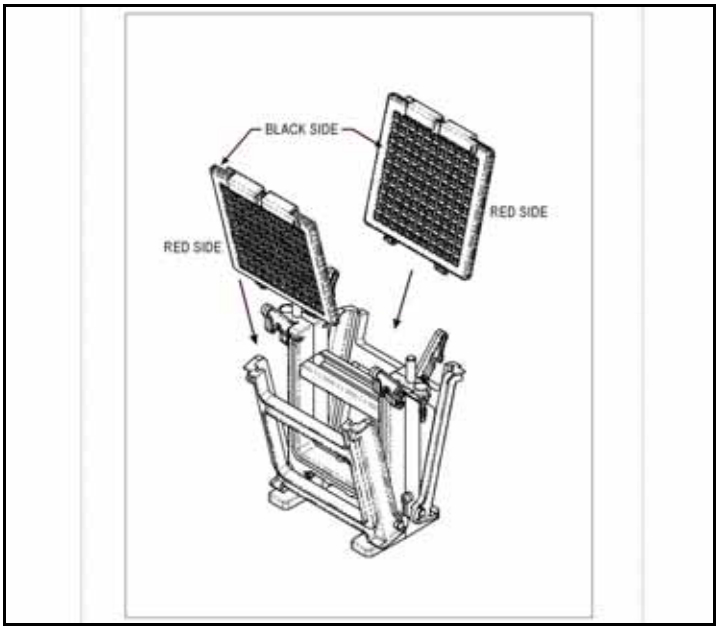
"Figure 3A: Opening Blotting Cassette"



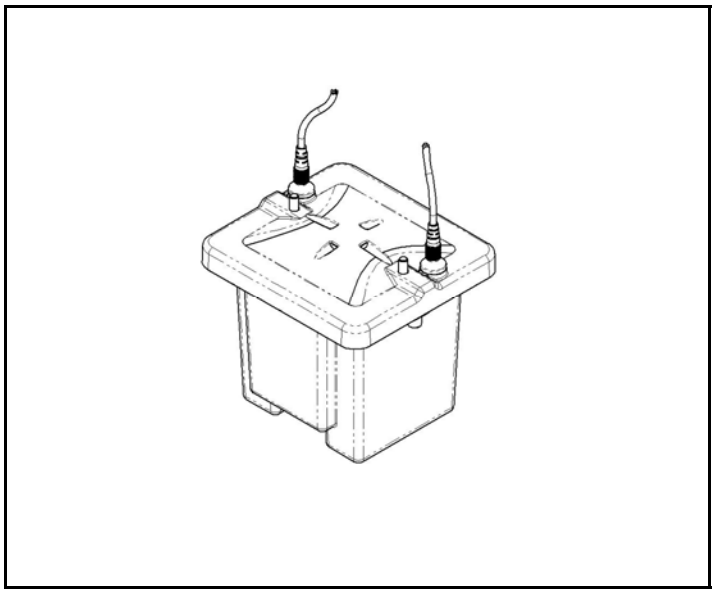
"Figure 3B: Assembly of Blotting Stack "



"Figure 3C: Insertion into Core Module."



"Figure 4: CosmoPAGE Dual Cell & Blot System – Closed"



4.3 Electroblotting

1. Fill the Lower Reservoir Assembly after the Core Module is inserted with 1x TGS Run/Blot Buffer (10x/20x #NU82500). The buffer should cover the blotting cassettes. The buffer should not come in contact with the banana plugs when the gel cassette sandwiches are immersed in the unit. With both ice blocks in place, about 1000ml of transfer buffer is required.

2. Align Safety Cover over the unit and carefully attach.

3. Connect the leads to the power supply, matching the color code: red to red and black to black.

4. When using recommended transfer buffer at about 22°C as above under 200VDC constant voltage transfer conditions with CosmoPAGE Gels, one gel will generate about 180mA and two gels about 215mA. When blotting with the buffer and apparatus at 4°C, the current will be much lower (about one-half) and the blotting time should be doubled.

5. Transfer times will vary according to several parameters. Optimization of electro-blotting transfers must be determined empirically. Keep in mind the following principles that govern the movement of proteins of gel electrophoresis:

   - Thicker or higher percentage gels will take longer to transfer than thinner or lower percentage gels.

   - Actual transfer times for defined conditions can be approximated by running pre-stained molecular weight standards.

4.4 Removing the Blotting Cassettes

1. Turn the power supply off and disconnect the leads from the power supply.

2. Remove the Safety Cover from the unit, by placing thumbs on white posts next to red and black connectors, then pushing down while pulling up with fingers under lid. **DO NOT pull on power cables.**

4. Gently lift the cassette from the unit. **Always wear gloves, eye protection and protective clothing.**

5. Mark the orientation of the membrane with a pencil or by cutting off a corner and take apart the blotting cassette carefully.

6. Process membrane.

7. Rinse all cell components **thoroughly** with de-ionized water. Immerse the buffer core in water.

SECTION 5

MAINTENANCE OF EQUIPMENT

5.1 Care and Handling

The plastic components of the CosmoPAGE Dual Cell & Blot System are fabricated from polycarbonate. Electrodes and connectors are made from pure platinum, stainless steel, and chrome-plated brass. As with any laboratory instrument, adequate care ensures consistent and reliable performance.

After each use, rinse buffer chamber, Core Module and Blotting cassettes **thoroughly** with de-ionized water. **The buffer core is best immersed in water to rinse buffer contaminants of the cavities.** Failure to properly rinse the buffers out of the cell components can lead to chemical attack and will void your warranty. 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, other non-water-soluble solvents, or undiluted alcohol.
- Avoid prolonged exposure of the apparatus or components to UV light, including sunlight.
- Do NOT treat with diethylpyrocarbonate (DEPC)-treated water for extended periods at 37°C. A brief rinse with DEPC-water is sufficient after a thorough wash, followed by a quick rinse in 70% ethanol.

5.2 Maintenance

The following inspection and maintenance procedures will help maintain the safety and reliable performance of the CosmoPAGE Dual Cell & Blot System. Replacement parts can be ordered by e-mailing sales@nacalaiusa.com, or by calling 858-404-0403.

- 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.