Azure Aqua™ Quad Mini-Cell

User Manual

Part Number AC4200

Rev 20200319

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1. General Overview

1.1 Introduction

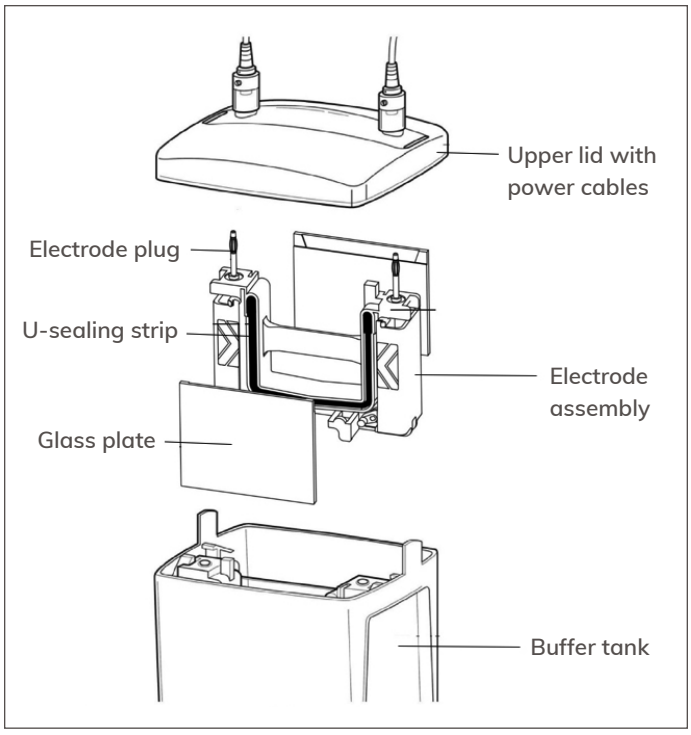
Azure Aqua Quad Mini-Cell is used to run up to four precast and hand-cast gels simultaneously. The system is compatible with 1-D and 2-D electrophoresis applications. The gel-casting frame and spacer plate are designed to make hand-casting easy while avoiding leakage.

1.2 Components

For optimal performance, please read the instruction manual carefully and operate the instrument according to recommended guidelines.

The following is a list of the Azure Aqua Quad Mini-Cell components:

- **Spacer Plate**—tall glass plate with fastening side strip. The glass plate is 1.0mm thick.
- **Short Glass Plate**—short glass plate that, when combined with the glass plate, forms the gel plate assembly.
- **Gel-Casting Frame**—blue casting frame with clamps. Place on a flat surface and align with the short glass plate and spacer plate to form the gel plate clamp.
- **Gel-Casting Stand**—clear stand for the Gel-Casting Frame
- **Gel Base**—the pressure lever seals the components of the gel apparatus to the gel casing pad to prevent leakage.
- **Replacement Plate for Single Gel**—transparent mound pressing baffle can be used to run one or three gels simultaneously.
- **Electrophoresis Cores**—holds the gel plate sandwich and includes the gasket, electrodes, and plugs. The anode is marked red and the cathode is marked black. Two cores are included – the primary core (identified by the electrode plugs) and the secondary core (without plugs).
- **Buffer Tank and Upper Lid**—compatible with both gel electrophoresis and transfer. Always firmly fix the lid in place before starting electrophoresis. After each run, ensure power is off prior to removing the lid.



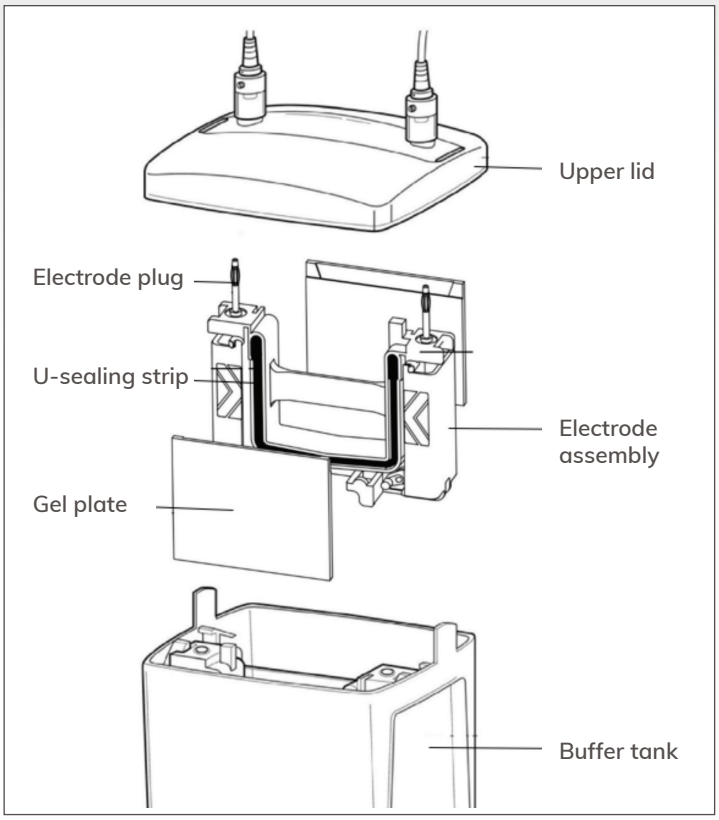


Figure 1. Gel electrophoresis parts and set-up.

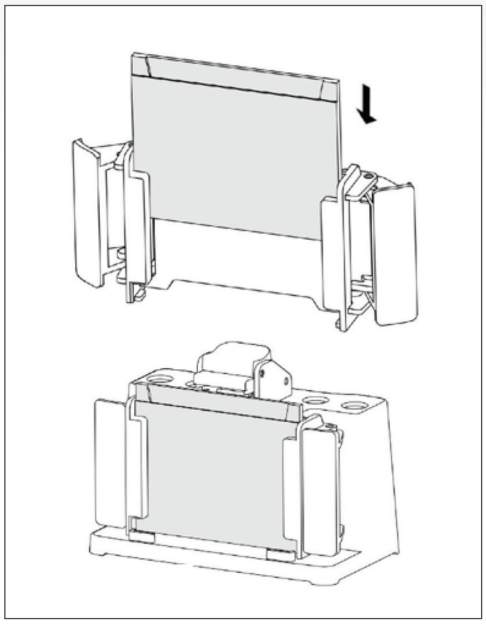


Figure 2. Gel-casting frame and gel-casting stand set-up.

1.3 Technical specifications

Maximum volume of samples:

Hole Quantity	Width of Hole	1.0 mm Gel Comb
10	5.08 mm	44 µl
15	3.35 mm	26 µl

Compatibility of chemical reagents:

All the components of the Azure Aqua Quad Mini-Cell should be kept clear of acetone and ethyl alcohol. Any damage caused by using one of these organic reagents is not covered by the warranty policy.

Be sure to keep the comb of the Azure Aqua Quad Mini-Cell away from 100%TEMED, which can cause damage.

1.4 Safety instructions

The power must be off before opening the upper lid. Never operate when the upper lid is not in place.

Note: *Be sure to follow all safety standards for the proper operation of all Azure products.*

2. Installation and Basic Operation

2.1 Gel casting

a. Preparing the asting frame and plates

1. Place the gel casting frame vertically on a flat surface and release the gel casting frame hinge.
2. Choose the appropriate spacer plate for the gel size you're using, and put the short glass plate above (Figure 3a)
3. Make sure the marked tip of the spacer plate is oriented up. Slide the short glass plate and the spacer plate into the gel casting frame (Figure 3b).

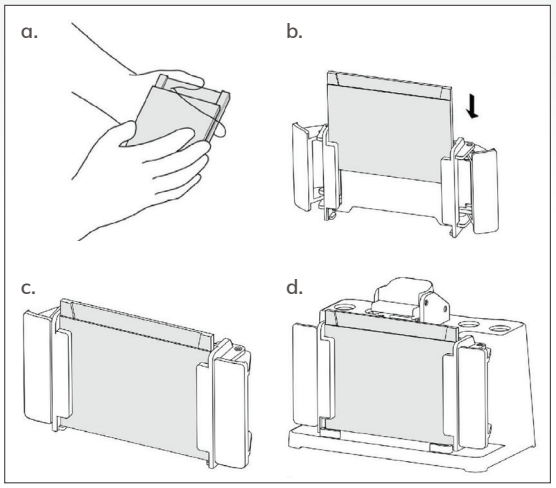


Figure 3. Installation of gel casting frame and gel base.

b. Basic instructions for casting discontinuous polyacrylamide gel

1. Place the comb into the assembled gel sandwich and mark the lane 1 cm below the comb teeth. This is the level to which you should pour the separating gel. Make sure to remove the comb after marking.
2. Mix all the reagents except APS and TEMED to make one solution and vacuum de-gas for at least 15 minutes.
3. Combine APS and TEMED after vacuum de-gassing and pour the solution between the glass plates until you reach the mark. Pour the solution slowly and consistently to avoid introducing air bubbles.
4. Cover the surface of the solution with water or tert-amyl alcohol (2- methyl,2- butanol). Be careful to avoid air bubbles.
5. Allow the gel to polymerize for 45-60 minutes. Clean the surface of the gel completely with ultra pure water. Do not leave any alcohol on the gel more than an hour in order to avoid dehydrating the upper part of gel.

   Note: *The gel can be stored at room temperature overnight. To store the gel, add 5 ml of 1.5MTri-HCl with ratio of 1 to 4 and buffer with Ph8.8 (Laemmli system) to the separating gel to be dried. If you are using a different buffer, add 5 ml of diluted solution.*
6. Dry the surface of separating gel with filter paper before pouring the stacking gel.
7. Prepare stacking gel solution - mix all of the reagents except APS and TEMED. Vacuum de-gas for at least 15 minutes.
8. Add APS and TEMED to the de-gassed spacer gel single solution and inject the solution until it reaches the level of the glass plate. Make sure there are no air bubbles.
9. Insert comb and make sure that the teeth are covered with solution.
10. Leave the gel for 45–60 minutes until it is polymerized.
11. Remove the comb slowly and wash the surface of gel with distilled water and buffer.

12. Clean the used gel clamp frame and gel casting frame with distilled water and deionized water.

c. Gradient polyacrylamide gel

1. Blend all of the reagents except APS and TEMED to make the gel solution and vacuum degass for at least 15 minutes.
2. After vacuum degassing, combine APS and TEMED and add the solution until it reaches the level of the glass plate.
3. Insert comb and make sure that the back of the comb and the glass plate are on the same level.
4. Leave the gel for 45–60 minutes until it is polymerized.
5. Take out the comb slightly and wash the surface of gel with distilled water and buffer.
6. Clean the used gel clamp frame and gel casting frame with distilled water and deionized water.

2.2 Running a gel

Note: *The electrophoresis core can be used for one or two gels. To run three or four gels, use the secondary core along with the primary core. Each module can hold up to two gels.*

1. Place the primary electrophoresis core on a flat surface and pull on the side locks to slide open (Figure 4a).
2. Place one gel on one side of the electrophoresis core. Place a second gel on the other side, pressing down on each gel firmly to ensure good contact with the bottom clips of the frame. If running a single gel, use the buffer dam in place of the second gel (Figure 4b).

   Note: *Orient the gel or buffer dam towards the center of the electrophoresis core (Figure 4b).*

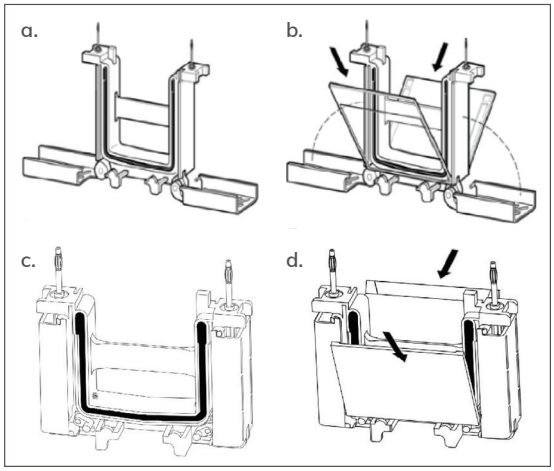


Figure 4. Electrophoresis module. The primary electrophoresis core can be used for one or two gels. To run three or four gels, use the secondary core

3. Make sure the gel or buffer dam are facing toward the center so the U-sealing strip has a tight seal.
4. Push the clamps closed tightly to prevent leakage (Figure 4c).

   Note: *Never try to close the core clamps if the gel plate does not fit properly beneath the U-sealing strip. This may cause leakage during electrophoresis. In order to prevent the gel plate from moving when you lock the apparatus, make sure the gel plate is pressed by the gel clamp firmly and evenly.*

5. Place the electrophoresis core in the tank and fill the upper chamber with running buffer. If running more than two gels, place the companion assembly in the back half of the tank.

   Note: *Do not place the secondary electrophoresis core in the cell unless running three to four gels to prevent extra heat from being generated which will affect electrophoresis.*

a. Put the Azure Aqua Quad Mini-Cell tank on a flat surface. Make sure the front is facing forward. You should see the red mark on the edge of the tank on your right and the black mark on your left.

b. When running two gels, use the main electrophoresis core (with electrode plugs). Match up the red (+) mark on the core to the red mark on the side of the tank

c. When running four gels, use the electrophoresis core with the electrodes and the companion core. Make sure to match the red(+) marks on both cores with the appropriate side of the tank.

Note: *If the cores are not placed correctly, the upper lid will not close.*

6. Adding samples:

a. Be careful not to overload the wells.

b. Only add the sample after the core is placed in the tank.

c. Pipette the sample into the well.

Note: *Pipette the sample slowly and evenly so it falls into the bottom of the well.*

Note: *Anode and cathode should be level and completely submerged in buffer.*

7. Fill the tank to the appropriate line for the number of gels being run.

a. Add buffer to the tank until it reaches the appropriate fill mark for the number of gels being run.

Note: *The buffer volume required for two gels is 540 mL and four gels is 1100 mL.*

8. Staring electrophoresis:

a. Align the electrode plugs on the electrophoresis core with the sockets on the upper lid of the tank and place the lid firmly on the tank.

Note: *The two tabs on the side of the buffer tank should fit through the slots in the upper lid.*

b. Insert the leads into the power supply by matching the colors to the sockets.

c. Start the electrophoresis run by switching on the power from the power supply. A constant voltage of 200V is recommended for SDS-PAGE and most of native PAGE. 200V is sufficient to run up to four gels.

9. After run completion:

a. When the run is complete, turn off power to the unit and unplug the leads from the power supply.

b. Remove the lid and carefully lift out the electrophoresis core. Pour out the buffer in the upper chamber before opening the clamp.

c. Remove the gel(s).

d. Carefully separate the glass or plastic plates and remove the gel.

e. If using hand cast gels, place the gel facing down and cover the gel and glass plate with buffer to help separate them.

f. Clean the Azure Aqua Quad Mini-Cell electrophoresis core and tank with ultra pure water.

3. Troubleshooting

Problem	Cause	Solution
Smile effect – band pattern curves upward at both sides of the gel	Center of the gel is running hotter than the ends because there is too much power. Or, the buffer may not be mixed well or the buffer in the upper chamber is too concentrated.	Decrease the power setting from 200 V to 150 V or fill lower chamber to within 1 cm of top of the short plate. Remake the buffer. Be sure to mix thoroughly, especially when diluting 5x or 10x stock.
Vertical streaking of protein	Sample is overloaded.	Dilute the sample, selectively remove the predominant protein in the sample, or reduce the voltage about 25% to minimize streaking.
Lateral band spreading	Diffusion of the wells prior to turning on the current. Ionic strength of the sample lower than that of the gel.	Minimize the time between sample application and turning on the power. Use the same buffer in sample as in the gel or the stacking gel.
Skewed or distorted band	Poor polymerization around wells, salts in sample, or uneven gel interface.	De-gas stacking gel solution prior to casting, increase ammonium persulfate and TEMED concentrations by 25%, for stacking gel or low gel concentration, leave APS the same and double the TEMED concentration. Remove the salts by dialysis, desalting or column. Overlay gels very carefully.
Lanes constricted at the bottom of the gel	Ionic strength of sample higher than the strength of the surrounding gel.	Desalt sample and neighboring samples.
Run taking unusually long	Running buffer too concentrated or excessive salt in sample.	Check buffer protocol. Dilute if necessary. Desalt sample.
Run too fast	Running or reservoir buffer too dilute or voltage too high.	Check buffer protocol. Dilute if necessary. Decrease voltage and re-try.
Doublets observed where single protein species is expected (SDS-PAGE)	A portion of the protein may have been reoxidized during the run or may not have been fully reduced prior to the run.	Prepare fresh sample buffer solution if over 30 days old. Increase concentration in the sample buffer. Substitute DTT for BME.
Fewer bands than expected and one heavy band at the dye front	Protein(s) migrating to the dye front or protein degradation.	Increase the gel concentration of the resolving gel. Use protease inhibitors, e.g., PMSF, etc.

Problem	Cause	Solution
Upper buffer chamber leaking	Upper buffer chamber overfilled or improper assembly.	Keep buffer level below the top of the spacer plate. Be sure gasket is clean, free of cuts, and lubricated with buffer. Be sure the short plate is under the notch on the gasket, not on top of it.
Leaking during hand casting	Chipped glass plates, spacer plate and short plate not level. Casting stand gasket is dirty, flawed, or worn out.	Ensure glass plates are not damaged and aligned correctly. Wash the gasket if it is dirty, replace casting stand gaskets if damaged or worn out.
Webbing/excess acrylamide behind the comb	Incorrect catalyst concentration.	Prepare fresh catalyst solution or increase the catalyst concentration of the stacking gel to 0.06% APS and 0.12% TEMED.
The pressure lever on the casting frame are difficult to close or make noise when closed	Powder residue has built up at the pivot of the pressure cams.	Rinse or wipe off the powder residue before each use.

4. Warranty Policy

Standard 1 year warranty. For more information visit https://www.azurebiosystems.com/warranty/