

SOFTRON

Oxygen Binding–Dissociation Analyzer

Bloodox-2019

Operating Manual



Before Using This Manual

This manual applies to the Bloodox-2019 Oxygen Binding–Dissociation Analyzer. It covers instrument installation, oxygen electrode preparation, software connection, test procedures, cleaning, maintenance, and troubleshooting. Read the entire manual before operating the system for the first time, and follow the procedures in the specified order during subsequent tests.

IMPORTANT SAFETY INFORMATION: This system uses compressed gas cylinders, nitrogen, and electrical components. Operate it only in a well-ventilated area and comply with your institution's compressed-gas and laboratory safety procedures. A substantial nitrogen leak may create an oxygen-deficient atmosphere.

PARAMETER NOTE: The recommended values in this manual are intended for routine testing. Confirm the appropriate settings for the sample type, laboratory environment, and study protocol, and keep all critical conditions consistent throughout the same study.

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1 Safety Requirements and Operating Environment

1.1 Unpacking Inspection

The instrument is inspected and packed before shipment. Upon receipt, examine the outer packaging for crushing, damage, or moisture. If any abnormality is found, retain the packaging, take photographs, and contact the carrier and supplier promptly. After unpacking, check the main unit, accessories, and cables against the packing list.

1.2 Operating Environment

Item	Requirement
Ambient temperature	15-40 °C
Relative humidity	85% or less, non-condensing
Power supply	220 V, 50 Hz, properly grounded
Installation site	Stable, level, and well ventilated; away from strong vibration, electromagnetic interference, and corrosive gases

1.3 Safety Precautions

- Secure each gas cylinder properly and fit it with a suitable two-stage pressure regulator. Do not use fittings or tools contaminated with oil or grease.
- Maintain the outlet pressure of both nitrogen and compressed air at 0.01-0.10 MPa. Never exceed 0.10 MPa.
- Before connecting or disconnecting any gas line, close the cylinder valve and release pressure from the line.
- Keep the work area well ventilated during operation. Do not allow nitrogen to accumulate in a confined or enclosed space.
- Do not allow samples, buffer, or cleaning solution to enter electrical connectors or the instrument enclosure.

- Do not disassemble the instrument except as required for cleaning, membrane replacement, and the routine maintenance described in this manual.

2 System Overview

2.1 Intended Use

The Bloodox-2019 records oxygen equilibrium curves for hemoglobin-containing samples. It can generate an oxygenation curve during oxygen binding and an oxygen dissociation curve during oxygen release. Suitable samples include whole blood, red blood cell lysates, and hemoglobin preparations.

2.2 Basic Workflow

1. Mix the hemoglobin-containing sample thoroughly with buffer and load it into the sample chamber.
2. Start heating and stirring, then introduce air to fully oxygenate the sample.
3. After the temperature and oxygen partial pressure have stabilized, calibrate the optical and PO₂ channels.
4. Introduce nitrogen to deoxygenate the sample gradually. The software simultaneously records PO₂ and optical absorbance changes and plots the oxygen dissociation curve.
5. To record oxygenation, switch to air after deoxygenation and acquire the oxygenation curve.

Under typical conditions, sample oxygenation and temperature stabilization take approximately 15-25 minutes. Recording a single curve usually takes approximately 10-15 minutes. Actual times depend on the sample type, gas flow rate, temperature, and other test conditions.

2.3 System Components



Figure 1. Main unit and principal components

The main unit has a metal enclosure and a touch-control panel on the right. The transparent top window opens to provide access to the sample chamber. The chamber contains a Clark-type oxygen electrode, a temperature sensor, and a magnetic stir bar; a magnetic stirrer is located beneath the chamber. The port at the bottom of the chamber serves as both the gas inlet and the liquid drain.

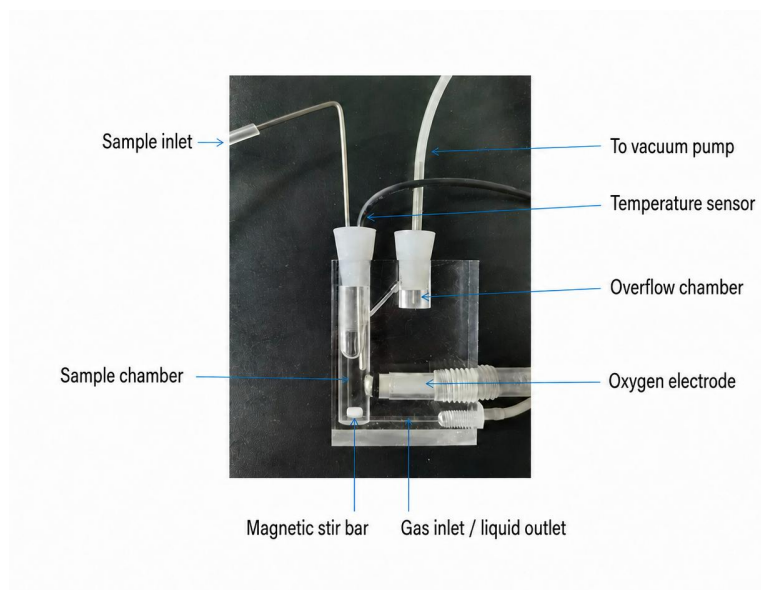


Figure 2. Sample chamber and connection points

3 Installation and Connections

3.1 Power and Data Connections

1. Place the main unit on a stable, level surface with sufficient access for operation.

2. With the power switch in the OFF position, connect the instrument to a properly grounded 220 V, 50 Hz power supply.
3. Connect the main unit to the computer using the USB-to-serial cable.

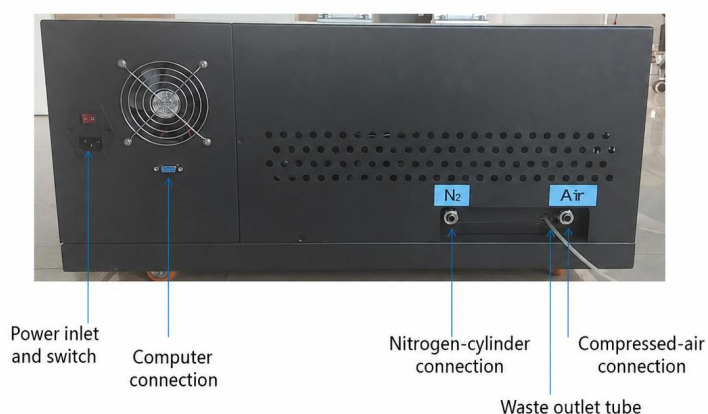


Figure 3. Rear-panel connections

3.2 Gas Connections

The user must provide compressed nitrogen and compressed air. Nitrogen purity must be at least 99.99%. Fit each cylinder with a two-stage pressure regulator. The supplied tubing has an inside diameter of 7.5 mm and an outside diameter of 10 mm. Connect the lines to the rear-panel ports marked N₂ and AIR, respectively.

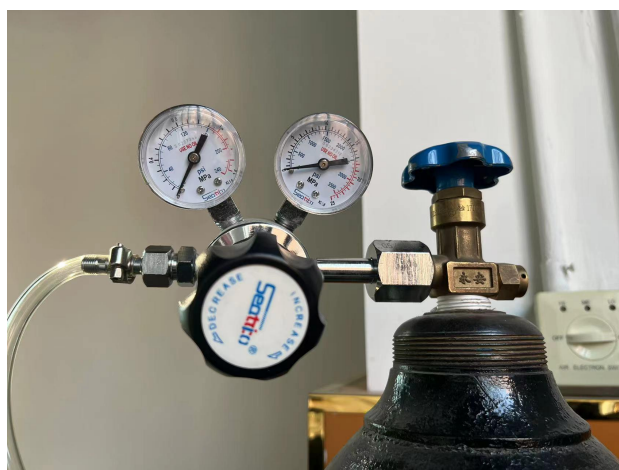


Figure 4. Gas-cylinder pressure regulator

1. Turn the adjustment knob on the second-stage regulator counterclockwise until the regulator is closed or set to its minimum output.
2. Slowly open the main cylinder valve.

3. Turn the second-stage regulator clockwise until the outlet pressure stabilizes within 0.01-0.10 MPa.
4. Install the lever valve in the nitrogen line approximately 10 cm from the instrument, and check all connections for leaks.

WARNING: Do not allow the gas outlet pressure to exceed 0.10 MPa. Excessive pressure may damage the instrument's internal gas-path components.

3.3 Waste-Line Connection

Insert the waste tube from the rear panel into a waste container compatible with the sample and cleaning solutions. Position the tube so that its end cannot become blocked as the liquid level rises. Dispose of waste in accordance with applicable laboratory procedures and local requirements.

3.4 Purging the Nitrogen Line

After installation, purge air from the nitrogen line with high-purity nitrogen.

1. Switch on the instrument, open the parameter settings screen, and temporarily set the N₂ flow rate to 20 mL/min.
2. Return to the main screen and press N₂ to deliver nitrogen to the sample chamber.
3. Continue the purge for approximately 20-30 minutes. Then press N₂ again to stop the gas flow and restore the flow rate required by the test protocol.

Purge the nitrogen line again under any of the following conditions:

- When the nitrogen line is connected for the first time;
- After replacing the nitrogen cylinder; or
- Before resuming use after the main cylinder valve or regulator has remained closed for an extended period.

Operating tip: If the interval between tests is short, keep the cylinder outlet pressure within 0.01-0.10 MPa and close the lever valve in the nitrogen line to reduce backflow of air. For extended shutdown, close the main cylinder valve and release pressure from the line.

4 Clark-Type Oxygen Electrode

4.1 Construction and Function

The oxygen electrode consists of a platinum cathode, a silver anode, and an insulator between them. It is a Clark-type oxygen electrode. A gas-permeable polytetrafluoroethylene (PTFE) membrane covers the electrode surface, with saturated potassium chloride solution serving as the electrolyte between the membrane and the electrode. The electrode continuously measures the oxygen partial pressure (PO_2) in the sample and is suitable for recording dynamic PO_2 changes.

4.2 Installing the Oxygen-Permeable Membrane

1. Rinse the electrode surface with distilled water, gently shake off excess water, and make sure no residue remains.
2. Prepare a saturated potassium chloride solution using distilled water.
3. Hold the electrode with the tip facing upward. Using a pipette, place approximately 25 μL of saturated potassium chloride solution on the electrode tip to form a droplet.
4. When handling a new membrane, do not touch its central area. Place the membrane over the droplet, taking care not to allow electrolyte to enter the electrode connector.
5. Place a new O-ring on the installation tool. Use the tool to push the O-ring into the groove around the middle of the electrode, securing the membrane in place.
6. Adjust the membrane so that it lies flat against the cathode and insulator. Make sure there are no air bubbles beneath it and no visible wrinkles in the membrane.
7. Slowly insert the electrode into the sample chamber. Lightly wetting the chamber can reduce resistance and help prevent the O-ring from shifting.



Figure 5. Cleaning the oxygen electrode



Figure 6. Applying electrolyte and positioning the membrane



Figure 7. Installing the O-ring with the installation tool



Figure 8. Securing and adjusting the membrane



Figure 9. Completed membrane installation

CRITICAL CHECK: Electrode response and measurement stability depend directly on a flat membrane surface, the absence of trapped air bubbles, and correct sealing by the O-ring.

4.3 Storage

During Continuous Use

- Drain the sample and clean the sample chamber immediately after each measurement.
- After cleaning, fill the chamber with distilled water or buffer and leave it filled until the next test. Do not leave a sample in the chamber for an extended period.
- Whenever the membrane is replaced, inspect and clean the electrode surface and remove any deposits.
- If sample or buffer enters beneath the membrane or into the electrode channel, remove the electrode and clean it immediately.

At the End of Each Day

- Remove the electrode from the instrument.
- Remove the membrane and clean the electrode.
- Allow the electrode to air-dry, or gently blot it dry with absorbent paper.
- Fit the red protective cap and store the electrode in a dry container with desiccant.

4.4 Routine Maintenance and Service Life

After extended use, silver chloride or silver oxide deposits may form on the silver anode.

Perform the maintenance procedure below if the PO₂ reading becomes abnormal—for example, if it remains above 200 mmHg and does not decrease during nitrogen flow, or if it does not fall below 10 mmHg after 30-40 minutes of continuous nitrogen flow:

1. Apply a small amount of toothpaste to a soft-bristled toothbrush and gently brush the electrode tip.
2. Rinse thoroughly with clean water, making sure no toothpaste remains.
3. Allow the electrode to air-dry or blot it dry with absorbent paper, then fit the red protective cap.
4. Place the electrode in a dry container with desiccant, or in a dry cabinet at room temperature, for 24-48 hours.

Record the electrode's cumulative energized time and perform maintenance after approximately every 100 hours of energized operation. The electrode remains energized

whenever the main unit is switched on, even when no sample is being measured. Switch off the main unit or disconnect the electrode when measurements are not in progress.

Each electrode undergoes a 5-10-hour operational test before shipment and is vacuum packed. Under verification conditions using a bovine hemoglobin reference material, cumulative electrode use can exceed 500 hours. Actual service life depends on sample composition, energized time, cleaning and maintenance practices, and storage conditions.

5 Software Installation and Instrument Connection

5.1 Software Components

Software	Function
BL10	Connects to the main unit, acquires data, and plots curves in real time
BL10 Viewer	Opens, displays, and analyzes saved data

5.2 Installing the Driver and Software

1. Install the DriverInstaller USB-to-Serial driver on a Microsoft Windows computer.
2. Double-click BL10Setup and follow the on-screen instructions to install the software.
3. Connect the main unit to the computer using the USB-to-serial cable, then switch on the main unit.

5.3 Establishing Communication

1. Launch BL10.
2. Under Comm Ports, select the COM port assigned to the instrument.
3. Click Connect. Communication is established when the status indicator changes from red to green and Connected appears.
4. To close the software, click Exit.

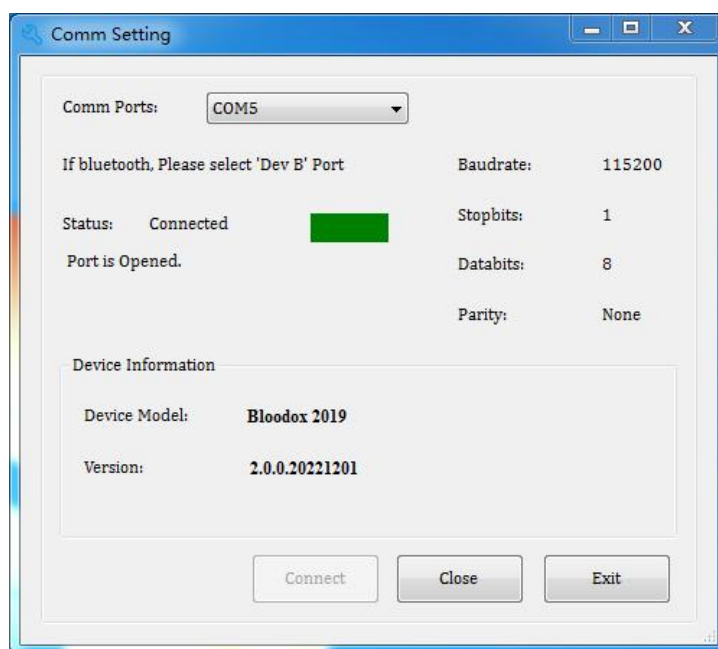


Figure 10. COM-port connection settings

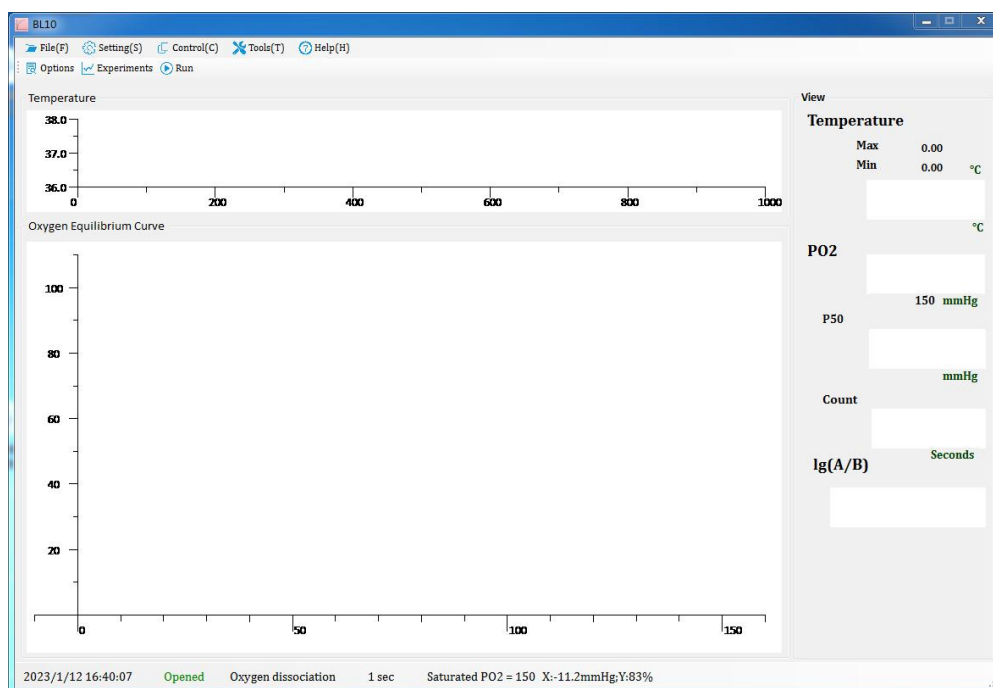


Figure 11. BL10 data-acquisition screen

6 Measurement Principles and Key Parameters

6.1 Optical Detection of Hemoglobin Oxygen Saturation

Oxyhemoglobin (HbO_2) and deoxyhemoglobin (Hb) have different absorption spectra. The system uses 570 nm as the reference wavelength and 560 nm as the measurement wavelength. As HbO_2 is converted to Hb , absorbance changes relatively little at 570 nm but

changes markedly at 560 nm. The system records this difference as the underlying signal for the vertical axis of the curve.

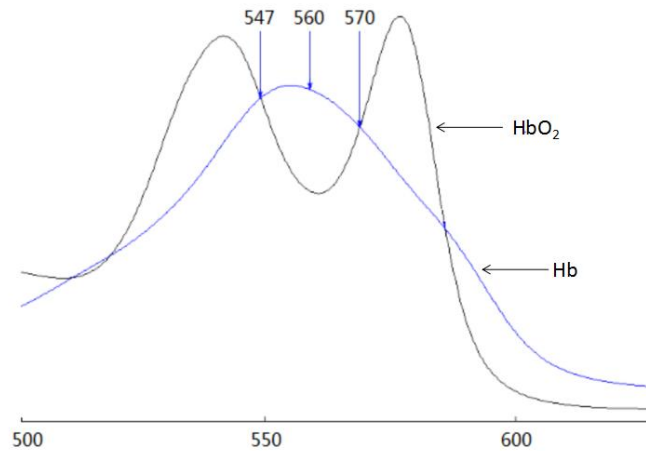


Figure 12. Absorption spectra of HbO₂ and Hb

6.2 Oxygen Partial Pressure Measurement

The system uses a Clark-type oxygen electrode to measure sample oxygen partial pressure directly. The PO₂ and optical signals are transmitted synchronously to the computer to generate the oxygen equilibrium curve.

6.3 Estimating Saturated PO₂ During Air Oxygenation

When air is used to oxygenate the sample, saturated PO₂ at 37 °C can be estimated using the following equation:

$$PO_2 = (P_{at\ m} - P_v) \times a$$

Symbol	Definition
$P_{at\ m}$	Actual atmospheric pressure at the test site, in mmHg
P_v	Water-vapor pressure at 37 °C: 47 mmHg
a	Volume fraction of oxygen in the oxygenating gas; use 0.21 for air

Example: At an atmospheric pressure of 760 mmHg with air oxygenation, $PO_2 = (760 - 47) \times 0.21 \approx 150$ mmHg. This value varies with altitude, weather, and gas composition; calculate it using the actual atmospheric pressure at the test site.

6.4 Key Operating Parameters

Parameter	Description and recommendation
PO ₂	Saturated oxygen partial pressure when air is used for oxygenation.
Temperature	Sample temperature. For simulated physiological conditions, the typical setting is 37 °C. If the measured temperature is slightly low or high, adjust the temperature compensation value in small increments. A typical compensation range is -1.0 to 1.0.
AIR	Air flow rate. Excessive flow may destabilize the curve; insufficient flow extends oxygenation time.
N ₂	Nitrogen flow rate. Excessive flow may destabilize the curve; insufficient flow extends deoxygenation time.
Recommended flow rate	For whole-blood measurements, select AIR and N ₂ flow rates within 6-10 mL/min and keep them constant throughout the same study.

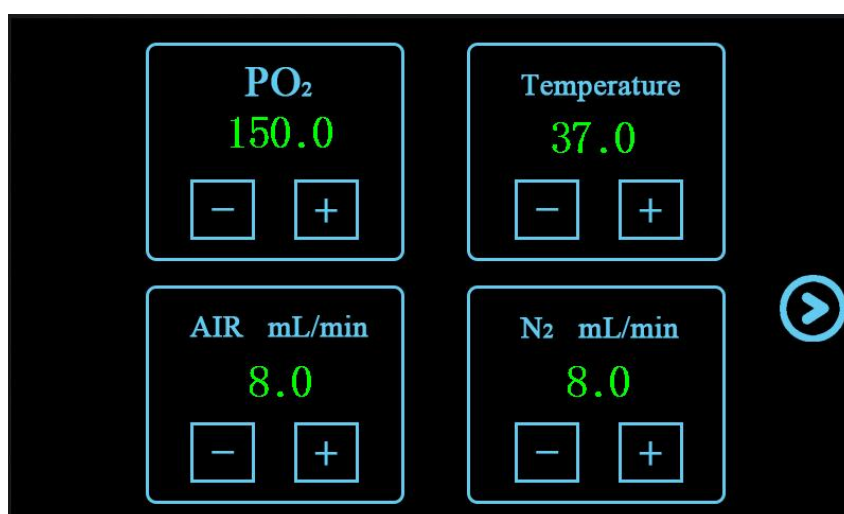


Figure 13. Temperature and gas-flow settings

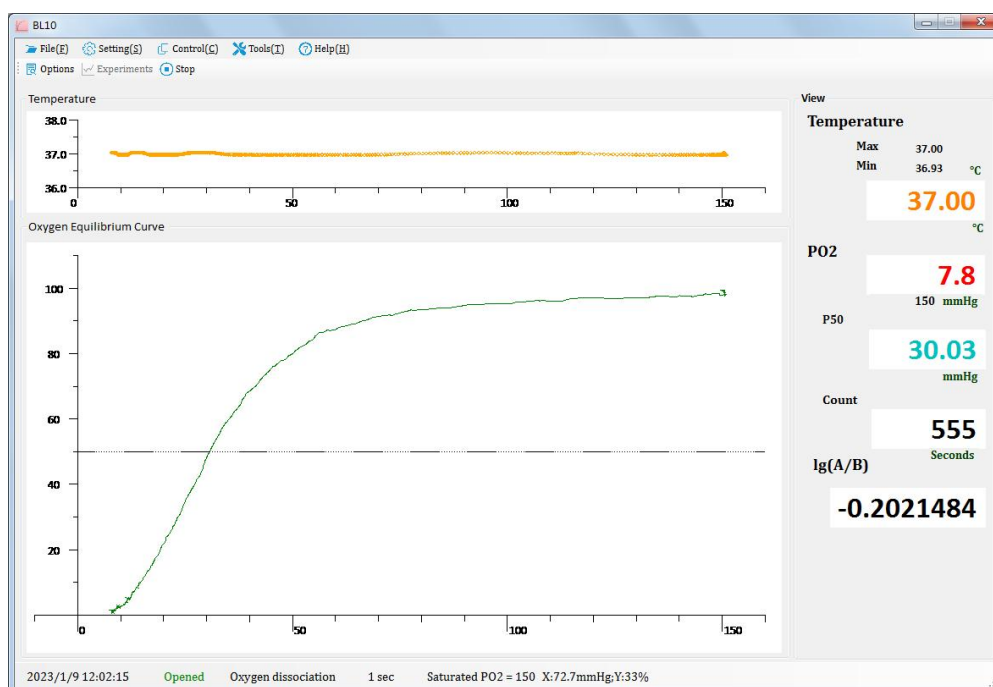


Figure 14. Typical oxygen dissociation curve

7 Pre-Test Checks and Membrane Conditioning

7.1 Checks Before Power-On

- Verify that the power cable, data cable, gas lines, and waste line are connected correctly.
- Verify that the oxygen-permeable membrane is installed correctly on the oxygen electrode and that the magnetic stir bar is in the sample chamber.
- Verify that the compressed-air and nitrogen cylinders are open and that their outlet pressure is 0.01-0.10 MPa.
- Verify that BL10 and the USB-to-Serial driver are installed.

7.2 Membrane Conditioning and System Check

1. Switch on the main power switch at the rear of the instrument. The screen shown in Figure 15 appears.
2. Press FILL to draw approximately 4 mL of distilled water or buffer into the chamber. Press FILL again to stop filling.
3. Press STIR to start heating and stirring.
4. Tap the  button on the touchscreen to open the parameter settings screen. Set saturated PO_2 , Temperature, AIR, and N_2 .

5. Press Lamp Power on the instrument control panel to switch on the light source.
6. In BL10, select Control > Preparation. Set N₂ to 20 min, Air to 10 min, and Repeat to 3, then click Start.
7. When the cycles are complete, read the PO₂ value on the touchscreen. A value of approximately 6 mmHg or lower indicates that the instrument and membrane are operating normally.
8. Press N₂ on the touchscreen to stop nitrogen flow, then prepare the sample for testing.

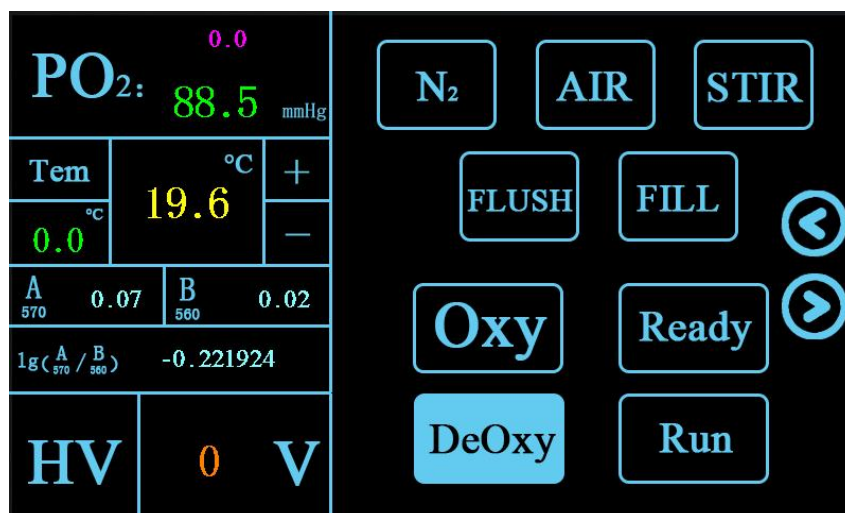


Figure 15. Main control screen

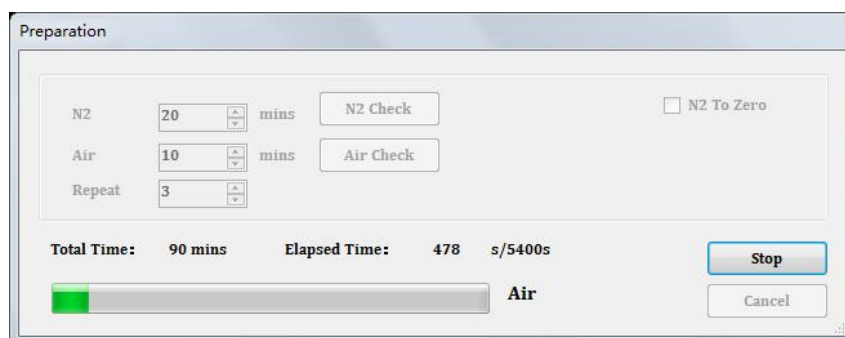


Figure 16. Preparation-program settings for membrane conditioning

MANDATORY: Condition the membrane and perform the system check after every membrane replacement. Running the procedure at the start of each test day is also recommended to confirm correct operation of the instrument, gas path, and membrane.

8 Sample Testing

8.1 Sample Preparation and Loading

1. Add 4 mL of buffer and an appropriate amount of sample to a centrifuge tube, then mix thoroughly. The prepared sample should contain approximately 3-6 mg of hemoglobin.
2. Press FLUSH to drain the liquid currently in the sample chamber.
3. Press FILL to draw a small amount of buffer into the chamber. Allow it to stand for several seconds, then press FLUSH to drain it. This step may be omitted if the liquid already in the chamber is the same buffer used for the current test.
4. Press FILL to draw the sample into the chamber. When the entire sample has entered, press FILL again to stop filling.

Whole-blood samples: To minimize the effect of negative-pressure aspiration on red blood cells, remove the plug from the sample inlet, slowly add the sample directly to the chamber, and then replace the plug securely.

8.2 Preparing the System for Measurement

1. Verify that PO₂, Temperature, AIR, and N₂ are set according to the test protocol.
2. Verify that the magnetic stir bar is in the sample chamber, then press Lamp Power to switch on the light source.
3. To acquire an oxygen dissociation curve first, select DeOxy and then Ready on the touchscreen.
4. When Ready is selected, AIR and STIR start automatically. All buttons except RUN are locked. To change a setting, cancel Ready first.
5. Wait for the PO₂ reading to stabilize gradually, and confirm that the sample temperature has reached and remains at the target value (typically 37 °C). This process usually takes approximately 15-25 minutes.

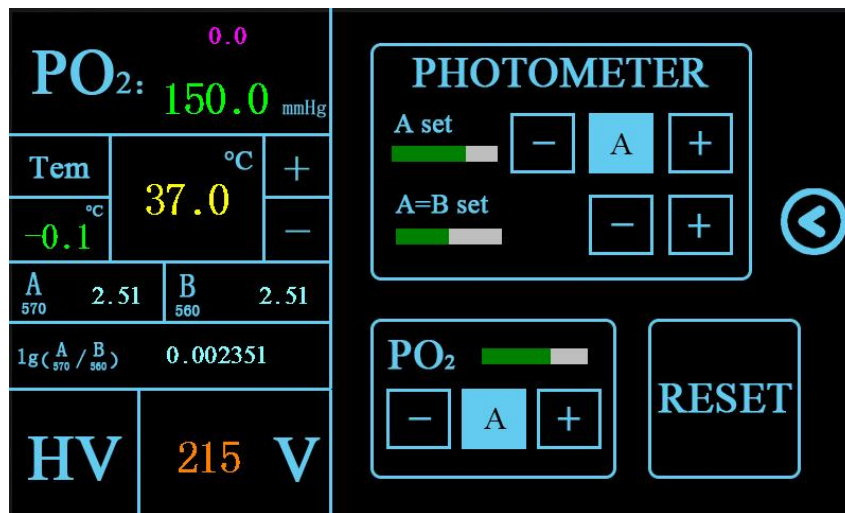


Figure 17. DeOxy/Ready status before measurement

8.3 Optical and PO₂ Calibration

After the sample is fully oxygenated and the temperature has stabilized, tap the  button on the touchscreen to open the calibration screen. Make the following adjustments in order:

1. Adjust A set until the displayed value is between 1.5 and 2.5. The HV value will typically be approximately -150 to -300.
2. Adjust A=B set until the two displayed values are as close as possible and their difference is close to 0 (0.00XXXX).
3. Adjust PO₂ until the PO₂ value in the upper-left corner of the main screen matches the saturated value calculated from the actual atmospheric pressure.
4. Tap  to adjust the value. After each adjustment, wait several seconds for the display to stabilize before continuing. Press RESET if necessary to restore the previous settings.

Figure 18. PO₂ calibration

8.4 Setting the Data-Save Location

1. In BL10, click Option.
2. Under Save File Path, set Operation Data Path.
3. Set Folder Name. The recommended format is \YYYYMMDD\.
4. Set Format. TXT is recommended.
5. Click Apply, then click OK to save the settings.

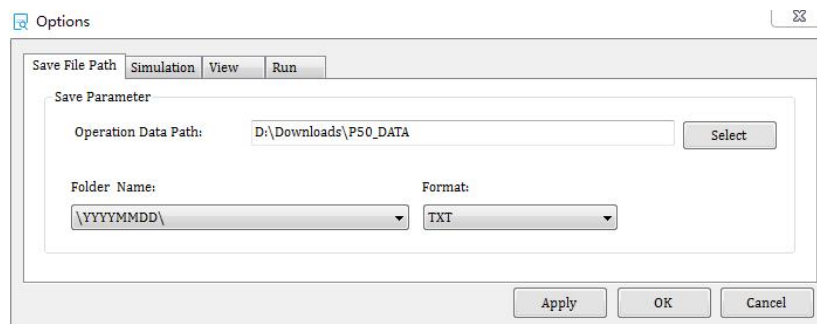


Figure 19. Data-save path settings

8.5 Acquiring an Oxygen Dissociation Curve

1. In BL10, click Experiments.
2. Under PO₂ Saturated, enter the saturated PO₂ value. This value defines the starting point of the oxygen dissociation curve.
3. Set Maximum Count. This is the maximum acquisition time in seconds. For example, if it is set to 1800, acquisition stops automatically when the count reaches 1800 seconds.
4. Enter the required sample identifier in Sample. Complete Study No and Comment as needed.

5. Click Start. N₂ starts automatically on the touchscreen, the gas supply switches from air to nitrogen, and the software begins recording the deoxygenation process in real time.
6. When PO₂ changes only slowly or stabilizes near the end of the curve, click Stop to end acquisition manually. Alternatively, allow acquisition to stop automatically when Maximum Count is reached.

Experiments

Connect Message
Message: Comm Port is opened. Device: Standby Comm

Device Setting
PO2 Saturated: 150 mmHg Maximum Count: 1800

Experimental Information
Sample: 20230112-002-Reference-30-02 Date: 2023/1/12 16:40:59
Study No: test
Data Path: D:\Downloads\P50_DATA\20230112
Comment:

Start Cancel

Figure 20. Experiment information settings

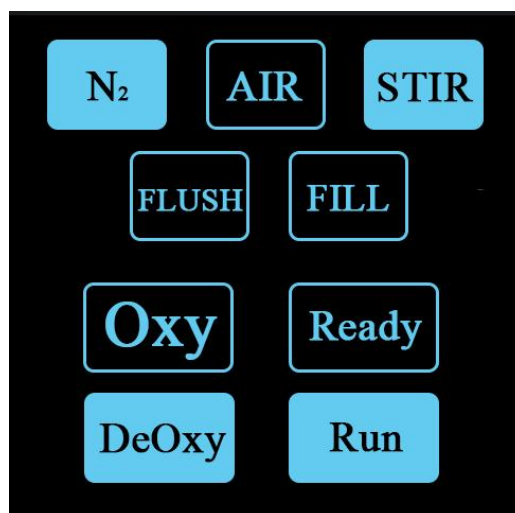


Figure 21. Main control screen during curve acquisition

8.6 Acquiring an Oxygenation Curve

1. On the touchscreen, select Oxy and then Ready. N₂ and STIR start automatically and bring the sample to the starting condition for the oxygenation curve.
2. In BL10, click Experiments and enter a new Sample identifier. Retain the confirmed PO₂ Saturated and Maximum Count settings if appropriate.
3. Click Start. The system switches automatically to air, and the software records the oxygenation process in real time.
4. Acquisition stops automatically and the oxygenation curve is generated when PO₂ reaches the specified saturated value.

8.7 Completing the Test and Cleaning the System

1. Switch off Lamp Power.
2. Press FLUSH to drain the sample chamber.
3. Press FILL to draw distilled water into the chamber, then press FLUSH to drain it. Repeat 3-5 times.
4. Finally, draw approximately 4 mL of buffer into the chamber and leave it in place during short intervals between tests.

Removing the sample chamber: If the instrument sounds an alarm after the chamber is removed, switch off Lamp Power or press RESET to restore the parameters. Before acquiring another curve, verify all settings again.

9 Maintenance and Troubleshooting

9.1 Main Unit Maintenance

- This is a precision instrument. Protect it from crushing, dropping, severe vibration, liquid ingress, and dust during transport and storage.
- Except during sample testing and the required warm-up or conditioning period, switch off the main unit to minimize cumulative operating time for the oxygen electrode, photomultiplier tube, halogen lamp, fan, and other components.
- Clean, maintain, and store the oxygen electrode as described in Section 4.

- Gas-line check valves may become contaminated as the number of tests increases. Replace them as necessary. During installation, make sure the arrow points in the direction of gas flow.



Figure 22. Sample-chamber area and gas-path components

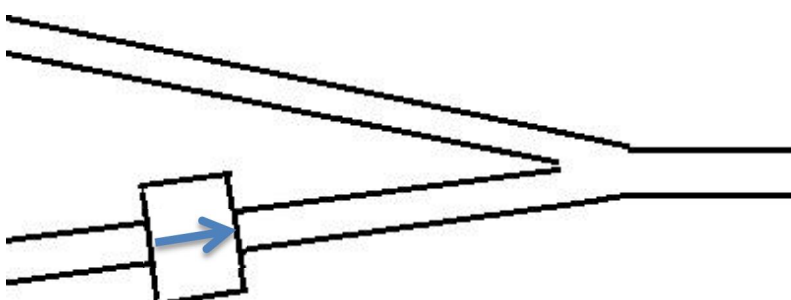


Figure 23. Check-valve installation direction

9.2 Troubleshooting

Symptom	Checks and corrective actions
BL10 cannot connect to the main unit	Verify that the main unit is switched on. Check the USB-to-serial cable and driver. Select the correct COM port again.

Symptom	Checks and corrective actions
PO ₂ decreases slowly or does not decrease during nitrogen flow	Check nitrogen purity, cylinder pressure, flow rate, gas-line leakage, and whether the nitrogen line was purged adequately. Inspect the membrane for air bubbles, wrinkles, or leakage. Service the oxygen electrode if necessary.
Temperature does not stabilize at the target value	Verify that STIR is operating. Check the ambient temperature. Adjust the temperature compensation value slightly and allow the reading to stabilize.
Curve fluctuates excessively	Check for excessive gas flow, improper stirring, air bubbles in the sample chamber, or an incorrectly installed membrane.
Poor drainage from the sample chamber	Check whether the waste line is kinked, blocked, or immersed too deeply in the waste container. Check the direction and condition of the check valve.

STOP USING THE INSTRUMENT if you notice an unusual odor, abnormal heating, liquid ingress into an electrical area, a significant gas leak, or a persistent alarm. Stop the test immediately, switch off the instrument, close the gas cylinders, and contact the supplier.

10 Contact Information

If the problem persists after completing the checks in this manual, record the instrument model, serial number, software version, test parameters, and observed abnormality.

Whenever possible, provide screenshots or curve files to assist technical support in diagnosing the problem.

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