INSTRUCTION MANUAL

MICRO-ePORE™

Pinpoint Cell Penetrator for targeted microinjection

Rev. 1 (For units with serial numbers up to 182508)

Serial No._____

www.wpiinc.com

052819

CONTENTS

ABOUT THIS MANUAL

The following symbols are used in this guide:

This symbol indicates a CAUTION. Cautions warn against actions that can cause damage to equipment. Please read these carefully.

This symbol indicates a WARNING. Warnings alert you to actions that can cause personal injury or pose a physical threat. Please read these carefully.

NOTES and TIPS contain helpful information.

Fig. 1—The MICRO-ePORE™ is designed for targeted microinjection.

INTRODUCTION

The new MICRO-ePORE™ pinpoint cell penetrator is a simple and versatile next generation targeted microinjection system. The system can be used for efficient microinjection of a diverse array of compounds and biomolecules into oocytes and pre-implantation stage of embryos in animal models including mice, rodents, zebrafish etc. The unique design of the MICRO-ePORE™ electrode holder allows for precise cell membrane penetration without tearing or damaging the cell membrane. MICRO-ePORE™ is suited for microinjection of CRISPR-Cas9 reagents into the cytoplasm of two-cell stage embryos, pronuclear zygote microinjection, and other gene manipulation – gene silencing and editing experiments.

The WPI MICRO-ePORE™ Pinpoint Cell Penetrator delivers a highly localized voltage signal to a targeted injection site to facilitate penetration with minimal trauma. The researcher determines the amplitude and frequency of the signal that best suits the application. Four sets of reconfigurable preset settings are provided to allow you to store and recall frequently used parameters. The signal originates in the control box, and it is transmitted through the electrode interface cable to the microelectrode holder. A chlorided silver wire is used to transmit the signal into the electrically conductive substance being injected. A reference electrode is used to place the media at 0.0 V potential with reference to the generated voltage.

To ensure proper injection, the MICRO-ePORE™ checks for continuity when it is not injecting. This ensures that injections are performed only when proper signal conduction is present by alerting you when there is not a viable current path to the specimen, which would make the signal application ineffective. Two warning levels are available:

- Continuous warning activates an audible alarm if no continuity is present. By default, alarms are disabled on power up.
- Momentary mode alert sounds with a distinctive tone when the inject pedal or the **Activate** button is pressed.

Features

- Touch-screen display–resistive touch panel for use with gloves
- Injection control through foot switch or manually through touch screen
- Intuitive user-interface
- User adjustable frequency and voltage through touch screen
- Small footprint
- Four user-programmable protocols
- Adjustable audio continuity tone indicating active probe
- Injection counter to indicate total number of injections
- Electrode holder compatible with most common microinjectors

Parts List

After unpacking, verify that there is no visible damage to the unit. Verify that all items are included:

(1) MICRO-ePORE™ controller

(1) Electrode holder with chlorided silver wire (Your choice of WPI #**300863** WPI MICRO-ePORE Holder, WPI #**300864** Femtojet® MICRO-ePORE Holder, or WPI #**300865** Narishige MICRO-ePORE Holder. Each electrode holder includes an electorde holder startup kits. See "Maintenance" on page 14 for details.)

(1) Microelectrode Holder Interface Cable

(1) Well-style Reference Electrode

(1) Probe-style Reference Electrode Assembly

(1) **13142** Foot Switch

(1) Power cord

(1) Instruction Manual

Unpacking

Upon receipt of this instrument, make a thorough inspection of the contents and check for possible damage. Missing cartons or obvious damage to cartons should be noted on the delivery receipt before signing. Concealed damage should be reported at once to the carrier and an inspection requested. Please read the section entitled "Claims and Returns" on page 19 of this manual. Please contact WPI Customer Service if any parts are missing at (941) 371-1003 or customerservice@wpiinc.com.

Returns: Do not return any goods to WPI without obtaining prior approval (RMA # required) and instructions from WPI's Returns Department. Goods returned (unauthorized) by collect freight may be refused. If a return shipment is necessary, use the original container, if possible. If the original container is not available, use a suitable substitute that is rigid and of adequate size. Wrap the instrument in paper or plastic surrounded with at least 100 mm (four inches) of shock absorbing material. For further details, please read the section entitled "Claims and Returns" on page 19 of this manual.

INSTRUMENT DESCRIPTION

Power Button

Fig. 2—The power button is on the front face of the MICRO-ePORE™ unit.

The home screen of the main display panel shows the current configuration of the unit. The Power button is located in the center of the front panel of the MICRO-ePORE™ unit.
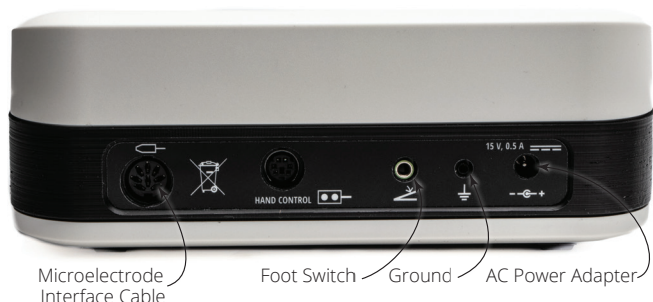

Microelectrode Interface Cable   Foot Switch   Ground   AC Power Adapter

Fig. 3—All the connections are located on the back panel of the MICRO-ePORE™ controller.

The back panel of the MICRO-ePORE has the following connnection ports:

Microelectrode Interface Cable–Connect the Microelectrode Interface Cable (probe cable) to this port. Be sure to line up all the pins and gently (but firmly) press the connector into the port.

Hand Control–This port is not used.

Foot Switch–Plug the foot switch connector into this jack.

Ground–If needed, this port gives you access to the ground potential. This may be used for connecting with other instruments to ensure a common ground reference. The MICRO-ePORE™ is grounded and the probe interface cable is shielded, so use of this port is optional.

AC Power Adapter–Plug the power supply into this port and connect the other end to an AC wall outlet.
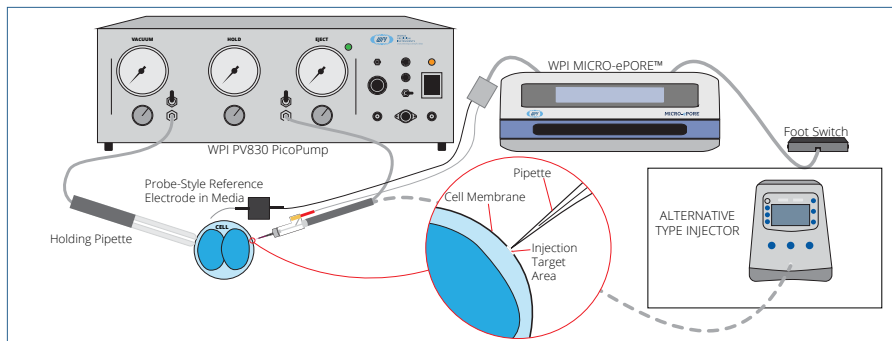
Setup

Fig. 4—A typical setup with the MICRO-ePORE™ using a probe-style reference electrode.
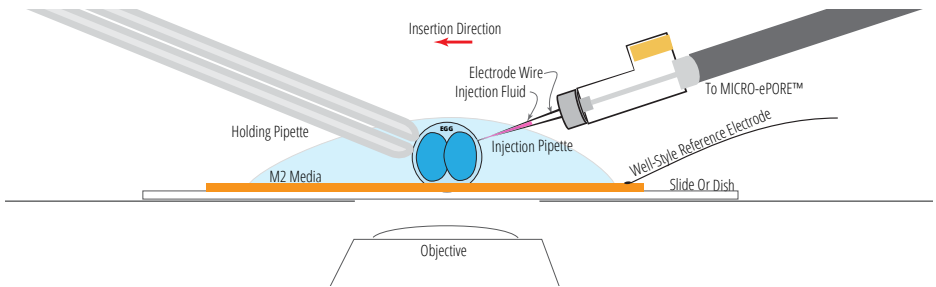
Fig. 5—The reference electrode ring is secured to a microscope slide with a vacuum gel, and the media is placed inside the well-style electrode. Both the probe-style electrode and the well-style electrode are included. See page 6. [Ring reference electrode – OD: 12.7 mm (0.5"), Thickness: 0.4 mm (0.16")]

Pipette Holder Connection

⚠️ **WARNING: GLASS PIPETTE SHOULD BE SECURED TIGHTLY BEFORE MAKING INJECTIONS.**

NOTE: The Pipette holder is designed to interface directly to popular injection systems like the WPI PV820/WPI PV830, Eppendorf Femtojet® or the Narishige injector.

1. The pipette holder has a silver wire that must be inserted into the glass micropipette. The wire must be long enough to make contact with the substance to be injected. This wire is intentionally left longer than most pipettes to allow for different lengths. Trim the wire to the preferred length.
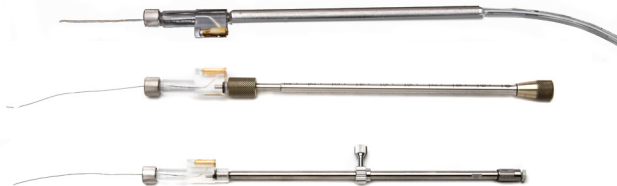

Fig. 6—Top to bottom, these pipette holders are designed for use with the WPI PV820 or WPI PV830, the Eppendorf Femtojet™, and the Narishige Injector.

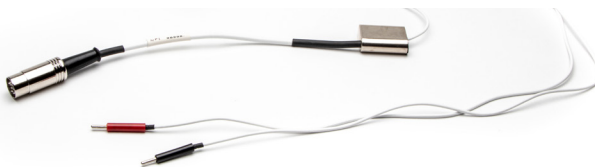2. The Microelectrode Interface Cable has two 2 mm male pin connectors.

Fig. 7—The Microelectrode Interface Cable has two connectors, one for the reference electrode and one for the microelectrode holder.

- The red pin connects to the 2 mm socket on the microelectrode holder. Insert the red pin into the socket on the microelectrode holder.

Fig. 8—The red pin of the microelectrode interface cable connects to the microelectrode holder.

- The black pin of the microelectrode interface cable connects to the 2 mm socket on one of the reference wires. This is necessary to complete the electrical connection to solution. For the well-style electrode, insert the black pin into the socket of the reference electrode (Fig. 9). For the probe-style electrode, the black pin of the microelectrode interface cable connects to the back side of the reference electrode block. The probe-style electrode pin plugs into the front of the block (Fig. 10).

Fig. 9—(Left) The well-style electrode is properly connected. [Ring reference electrode – OD: 12.7 mm (0.5"), Thickness: 0.4 mm (0.16")]
Fig. 10—(Right) The probe-style electrode is properly connected.

3. A. **Well-style electrode**–If you use the well-style electrode, the gold ring must be secured to your slide using vacuum grease to create an impermeable temporary seal. Place the liquid medium with your embryos inside the gold ring.

   B. **Probe-style electrode**–If you are using the probe-style electrode, place the block near the injection site. Then, bend the bare reference wire as needed to make contact with the liquid medium in order to complete the circuit.

World Precision Instruments

OPERATING INSTRUCTIONS

Understanding the Home Screen

When powered up, the controller starts up on the Home screen.
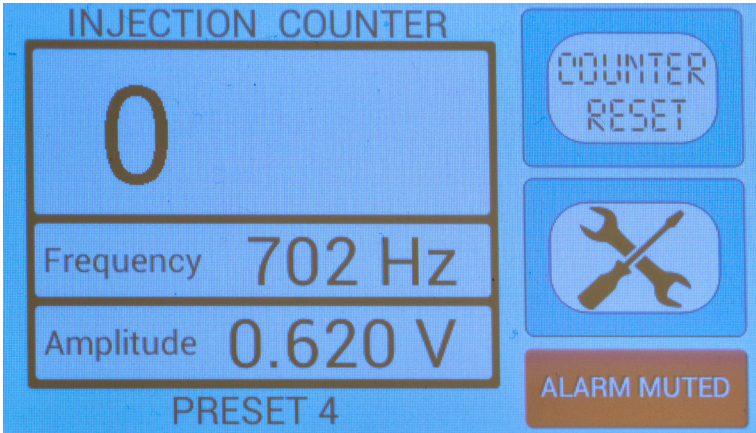
Fig. 11—The Home Screen shows the injection counter and the currently selected configuration parameters (frequency and amplitude).

The left side of the screen shows the injection counter, the frequency and the amplitude of each pulse initiated, and the current preset selection. The right side of the screen has three buttons.

- The top button resets the injection counter to zero.

- Press the middle button, the **Configuration** button, to access the Configuration screen.

- The bottom button toggles the alarms off and on. By default the alarms are disabled (red) on power up.

Understanding the Configuration Screen

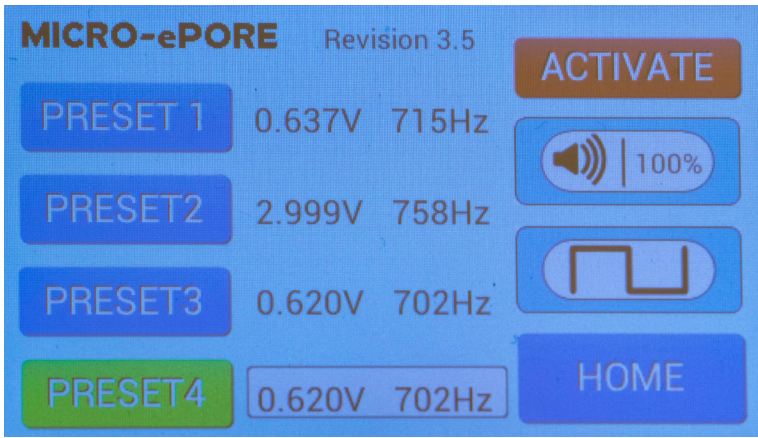Tap the **Configuration** button on the Home screen to access the Configuration screen.

Fig. 12—The Configuration Screen allows you to edit any of the presets.

The left side of the Configuration screen shows the four **Preset** buttons, and next to the **Preset** buttons is the description of the amplitude and frequency of each preset. The right side of the screen has four buttons:

- **Activate**–Press this button apply current through the micropipette. As long as you press the button (or the foot switch) current is applied using the parameters defined by the highlighted (green) preset.

- **Volume**–This toggle button sets the volume of the continuity alarm (100%, 75%, 50%, 25% or OFF).

- **Preset Configuration**–This button allows you to access the Preset Configuration Screen where you can set the amplitude and frequency for the highlighted (green) preset.

- **Home**–Press the **Home** button to return to the Home screen.

Preparing for an Injection

NOTE: The silver wire should be chlorided before use. MICRO-ePORE™ is provided with chlorided silver wire. If the chloride layer has worn out, please chloride the silver wire following the procedure in the Maintenance section of the Instruction Manual.

As you prepare your setup, use this checklist.

1. Check for continuity between the reference cable and the chlorided silver wire using this procedure:

    a. Connect the interface cable to the electrode holder and the reference cable. Use either the well-style reference electrode or the probe style reference electrode assembly.

    b. Turn on the audible alarm. (By default the alarms are silenced.) Simply press the red Alarm Muted button on the Home screen to enable the alarm.

    c. In the solution/buffer submerge both the silver wire of the electrode holder and the reference electrode.

    •   If the alarm tone is silenced, then you have continuity and all the connections are good.

    •   If the alarm sound continues, check all the connections.

    d. If you have continuity, you can disable the alarm by pressing the blue Alarm Enabled button on the Home screen..

    The background color of the injection counter indicates if a good connection to the specimen is achieved. (Green is good. Red indicates high resistance in the current path delivering the stimulus. In some cases, red indicates a possible issue.) This color change happens as the pedal is pressed and remains until the pedal is released.

2. Fill micropipette with injection solution. The silver wire should be in contact with solution.

3. Make sure that the right gasket is in the electrode-holder. Gaskets are color coordinated according to OD of glass being used.

4. Insert the micropipette into the electrode holder.

5. Temporarily enable the alarm and place the pipette and reference electrode in the media to verify continuity and eliminate other problems like bubbles. If the Alarm does not sound when the micropipette is in the solution, then you have continuity. The alarm can be disabled at your discretion. You are now ready to make injections.

6. Press the foot switch to activate the signal applied at the micropipette wire. You will hear a low frequency tone when the continuity on the probe is not adequate. Otherwise a steady tone is emitted.

NOTE: The reference wire is not always connected to ground. This is a multiplexed pin. Before pressing the pedal, this pin is connected to an input used to measure impedance in combination with the output wire (red terminal pin). When the pedal is pressed a measurement is taken, the reference pin is connected to ground and the output wire produces the stimulus signal.

Improving Cell Penetration

When using the MICRO-ePORE™ correctly, you should be able to perceive an improvement in the penetration of tissue. The first time you use the MICRO-ePORE™ you may need to adjust the voltage applied to optimize it for your particular application. WPI recommends increasing the voltage 10 mV at a time until the desired result is achieved.

Selecting a Preset Configuration

The unit comes with four user-definable preset configurations. You can choose the frequency and voltage (amplitude) for each preset. The period is determined by how long you press the foot switch (or the **Activate** button).

1. To select a preset, tap the **Configuration** button on the Home screen. The Configuration screen appears.
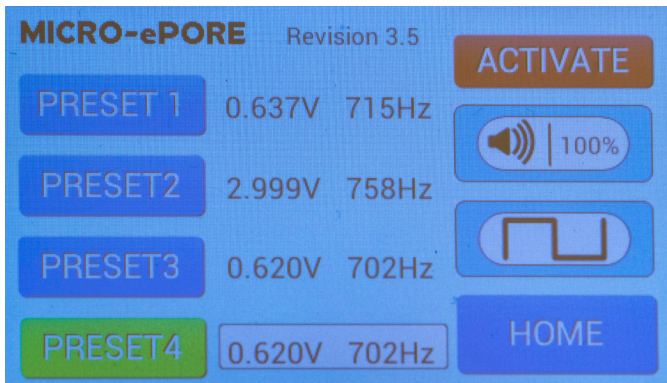

Fig. 13—Choose a preset on the left.

2. Tap the button for the preset you wish to use. A warning note displays. Tap the **Change** button to select the new preset or the **Cancel** button to keep the same preset and return to the Configuration screen.

Fig. 14—This warning appears when you select a new preset.

Editing a Preset Configuration

1. To change the parameters of a preset configuration, tap the **Configuration**
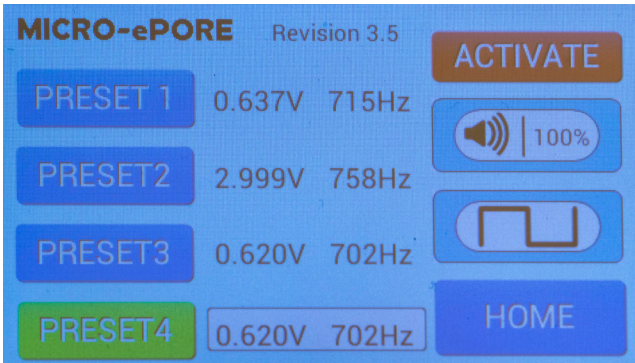   button on the home screen. The Configuration screen appears.

Fig. 15—The Configuration screen appears with the selected preset highlighted.
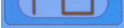
2. Select the **Preset Configuration** button to modify the selected
    preset. The Preset Configuration screen appears.

Fig. 16—From the Preset Configuration screen, you may define the frequency and amplitude.

3. To edit the frequency or amplitude, tap the parameter. Then, choose one of the following methods to edit the parameter:
    *   Use the **Increment** or **Decrement** button to change the parameter.
    *   Tap the **Keypad** button, enter the value for the parameter using the keypad and press **Enter**. Press the **Back** button to return to the Preset Configuration screen.
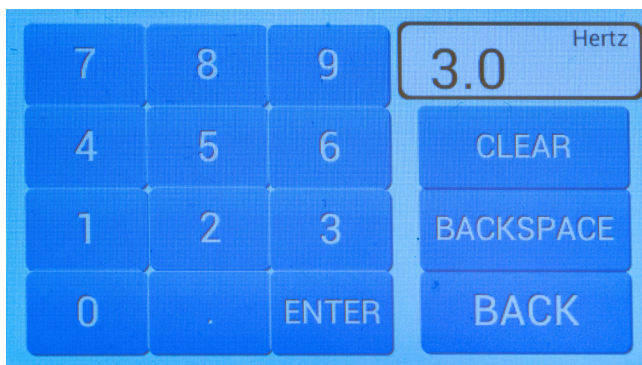

Fig. 17—Use the keypad to enter a value for a parameter and press Enter.

> **NOTE**: The setup is immediately saved to non-volatile memory. The parameter is remembered, ever after the unit is turned off.

4. Press **Done** to return to the Configuration screen.

> **NOTE**: From this screen, you may press the **Activate** button to begin applying current through the micropipette.

5. Press **Home** to return to the Home screen.

> **TIP**: The alarm sounds while your pipette is in the media. In rare cases the pipette opening may be extremely small, which adds a high resistance component to the overall stimulus path. This will cause the alarm to sound. The stimulus is always delivered when the pedal is pressed, regardless of the alarm state. However, if your application allows for a wider tip, you may open the pipette slightly. This would let you use the alarm feature.

Resetting the Injection Counter

Every time you press the foot switch (or press the **Activate** button on the

Configuration screen), current is applied through the probe in the micropipette. With each application, the injection counter increments by one. To reset the injection

counter to zero, press the **Counter Reset** button [COUNTER RESET] on the Home screen.

Muting Alarms

By default, alarms are disabled when you start the MICRO-ePORE™. If alarms are enabled, an alarm will sound when the circuit between the probe tip and the reference electrode is not complete. Without a complete circuit, the MICRO-ePORE™ will not be able to apply current.

To silence an alarm while you correct your setup error, press the **Alarm Enabled**

button [ALARM ENABLED] on the Home screen. The button turns red and changes to

Alarms Muted [ALARM MUTED].
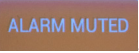
NOTE: Silencing the continuity alarm does not affect the tones that sound when the foot switch or **Activate** button is engaged.

NOTE: The alarm function does not affect the application of stimulus voltage. The stimulus will always be generated, regardless of the alarm state.

Adjusting Alarm Volume

1. To adjust the volume of the continuity alarm without turning it off, tap the **Configuration**

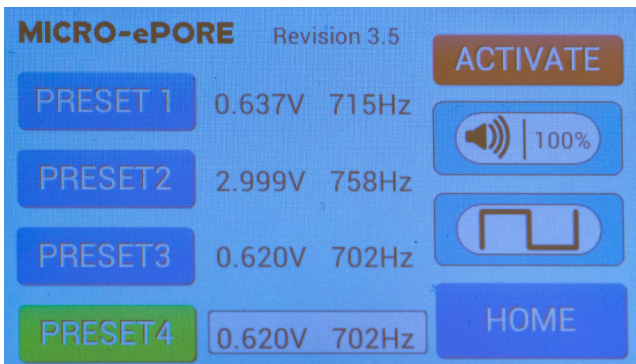button [⚒] on the home screen. The Configuration screen appears.

Fig. 18—The second button on the right side of the Configuration screen sets the alarm volume.

2. Select the **Alarm Volume** button to adjust the volume of the tones. This button toggles between 100%, 75%, 50%, 25% and OFF.

3. Press **Home** to return to the Home screen.

MAINTENANCE

Cleaning

The controller may be wiped down with a damp cloth to remove any biohazardous material. Then, wipe it with a paper towel and isopropyl alcohol.

> ⚠ **WARNING: DANGER! ELECTRIC SHOCK MAY RESULT DO TO THE INGRESS OF LIQUIDS.**
> - Power off the controller and disconnect the power cord before cleaning or disinfecting the unit.
> - Do not allow any liquids to penetrate the housing.
> - Do not spray cleaners or disinfectants on the housing.
> - Wait till the device is completely dry, inside and outside, before reconnecting the power.

> ⊘ **CAUTION**: Damage may result from the use of aggressive chemicals. Do not use any aggressive chemicals on the device or its accessories. Examples of aggressive chemicals include strong and weak bases, strong acids, acetone, formaldehyde, halogenated hydrocarbons and phenol. If the device has been contaminated by aggressive chemicals, clean it immediately using a mild cleaning agent.

Disinfecting/Decontaminating

Select disinfection methods that comply with the legal regulations and guidelines for your area of application.

Chloriding the Silver Wire

To chloride the silver wire, simply soak the clean wire in household bleach. Immerse the wire in full strength common household bleach, like Clorox, for 15 to 30 minutes until a purple-gray color is observed. Rinse before use.

ACCESSORIES

Part Number	Description
300683	WPI MICRO-ePORE™ Holder and startup kit*
300684	FEMTO MICRO-ePORE™ Holder and startup kit**
300685	NARISH MICRO-ePORE™ Holder and startup kit**
75122-110	Replacement gaskets, green, 1.0 mm, pkg. of 10
75122-210	Replacement gaskets, black, 1.2 mm, pkg. of 10
75122-310	Replacement gaskets, red, 1.5 mm, pkg. of 10
75122-410	Replacement gaskets, white, 1.65 mm, pkg. of 10
13142	Foot Switch
99192	Probe-style Reference Electrode
99190	Well-style Reference Electrode

*300683 startup kit includes:
  (1) PicoNozzle tip assemblies (Handle diameter is 6.25 mm and 100 mm long.)
  (1) 5' tubing
  (2) 1.0 mm pipette gaskets (lime green)
  (2) 1.2 mm pipette gaskets (black)
  (2) 1.5 mm pipette gaskets (red)
  (2) 1.65 mm pipette gaskets (white)
  (2) sealing gaskets

**300684/300685 startup kits include:
  (2) 1.0 mm pipette gaskets (lime green)
  (2) 1.2 mm pipette gaskets (black)
  (2) 1.5 mm pipette gaskets (red)
  (2) 1.65 mm pipette gaskets (white)
  (2) sealing gaskets

TROUBLESHOOTING

Issue	Possible Cause	Solution
An Alarm Sounds	Bubble formed in the pipette solution	Refill the pipette.
	Microelectrode Interface Cable is not properly connected	Check the Microelectrode Interface Cable and verify that it is properly connected.
	Non-conductive media	Ensure that the media you choose is electrically conductive.
	Reference electrode is not properly connected	Enable the alarms. Then, reposition the reference electrode until the alarm stops sounding.
	Reference electrode and the electrode holder are not in solution together	Reposition the reference electrode or the electrode holder so that both make contact with the solution to complete the circuit.
	Solution in the micropipette is not making contact with the electrode wire.	Refill the pipette with enough solution to cover the silver wire.
	A pipette with an extremely small tip diameter is placed in the media with the reference electrode, causing a high resistance reading in the overall stimulus path	The high resistance causes the MICRO-ePORE™ to give a false alarm signal. Regardless of the alarm state, the stimulus is always delivered when the pedal is pressed. However, if your application allows for a wider tip, you may open the pipette slightly. This would lower the resistance in the stimulus path and eliminate the false alarm signal, letting you use the alarm feature.
Screen on controller is dark	No power	Press the power button on the front of the control unit to power on the electronics.
	Power loss	Verify that the power supply is properly connected to the AC wall outlet and to the DC plug on the rear of the control unit.

NOTE: If you have a problem/issue with that falls outside the definitions of this troubleshooting section, contact the WPI Technical Support team at (941) 371-1003 or technicalsupport@wpiinc.com.

World Precision Instruments

SPECIFICATIONS

This unit conforms to the following specifications:

Voltage parameters.. 0–3.0 V, at 1 mV increments

Frequency parameters...50–3000 Hz, at 1 Hz increments

Pipette resistance alarm threshold maximum...500 MΩ

Dimensions .... 19.7 × 12.7 × 7.6 cm (7.75 × 5 × 3 in.)

Weight .... 0.9 kg (2 lb.)

Certifications.. CE, RoHS

Ring reference electrode OD..12.7 mm (0.5″)

Ring reference electrode thickness ....0.4 mm (0.16″)

DECLARATION OF CONFORMITY

WORLD PRECISION INSTRUMENTS, LLC.
175 Sarasota Center Boulevard
Sarasota, FL 34240-9258 USA
Telephone: (941) 371-1003 Fax: (941) 377-5428
E-mail: wpi@wpiinc.com

DECLARATION OF CONFORMITY CE

We: World Precision Instruments
                                    175 Sarasota Center Boulevard
                                    Sarasota, FL 34240-9258 USA

As the **manufacture/distributor** of the apparatus listed, declare under sole responsibility that the product(s):
Micro ePORE

To which this declaration relates is/are in conformity with the following standards or other normative documents:

Safety:
> **EN 61010-1:2010**

EMC:
> **EN 61326-2-3:2013**
> **EN 61326-1:2013**
> **EN 61000-3-2:2014**
> **EN 61000-3-3:2013**

And therefore conform(s) with the protection requirements of Council Directive 2014/30/EU relating to electromagnetic compatibility and Council Directive 2014/35/EU relating to safety requirements and Council Directive 2011/65/EU relating to hazardous substances:

Issued on: **February 12, 2019**

President of WPI

F-QC-006   Rev C

WARRANTY

WPI (World Precision Instruments) warrants to the original purchaser that this equipment, including its components and parts, shall be free from defects in material and workmanship for a period of 30 days* from the date of receipt. WPI's obligation under this warranty shall be limited to repair or replacement, at WPI's option, of the equipment or defective components or parts upon receipt thereof f.o.b. WPI, Sarasota, Florida U.S.A. Return of a repaired instrument shall be f.o.b. Sarasota.

The above warranty is contingent upon normal usage and does not cover products which have been modified without WPI's approval or which have been subjected to unusual physical or electrical stress or on which the original identification marks have been removed or altered. The above warranty will not apply if adjustment, repair or parts replacement is required because of accident, neglect, misuse, failure of electric power, air conditioning, humidity control, or causes other than normal and ordinary usage.

To the extent that any of its equipment is furnished by a manufacturer other than WPI, the foregoing warranty shall be applicable only to the extent of the warranty furnished by such other manufacturer. This warranty will not apply to appearance terms, such as knobs, handles, dials or the like.

WPI makes no warranty of any kind, express or implied or statutory, including without limitation any warranties of merchantability and/or fitness for a particular purpose. WPI shall not be liable for any damages, whether direct, indirect, special or consequential arising from a failure of this product to operate in the manner desired by the user. WPI shall not be liable for any damage to data or property that may be caused directly or indirectly by use of this product.

Claims and Returns

Inspect all shipments upon receipt. Missing cartons or obvious damage to cartons should be noted on the delivery receipt before signing. Concealed loss or damage should be reported at once to the carrier and an inspection requested. All claims for shortage or damage must be made within ten (10) days after receipt of shipment. Claims for lost shipments must be made within thirty (30) days of receipt of invoice or other notification of shipment. Please save damaged or pilfered cartons until claim is settled. In some instances, photographic documentation may be required. Some items are time-sensitive; WPI assumes no extended warranty or any liability for use beyond the date specified on the container

Do not return any goods to us without obtaining prior approval and instructions from our Returns Department. Goods returned (unauthorized) by collect freight may be refused. Goods accepted for restocking will be exchanged or credited to your WPI account. Goods returned which were ordered by customers in error are subject to a 25% restocking charge. Equipment which was built as a special order cannot be returned.

Repairs

Contact our Customer Service Department for assistance in the repair of apparatus. Do not return goods until instructions have been received. Returned items must be securely packed to prevent further damage in transit. The Customer is responsible for paying shipping expenses, including adequate insurance on all items returned for repairs. Identification of the item(s) by model number, name, as well as complete description of the difficulties experienced should be written on the repair purchase order and on a tag attached to the item.

Electrodes, batteries and other consumable parts are warranted for 30 days only from the date on which the customer receives these items.