WORLD
PRECISION
INSTRUMENTS

INSTRUCTION MANUAL

# CardioPhys™ ECG

*Electrocardiogram Monitoring System*

Serial No._____

www.wpiinc.com

100319

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# 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.

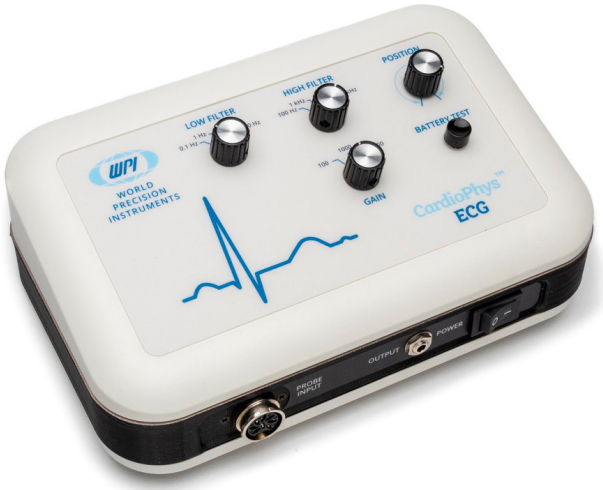


*Fig. 1—The CardioPhys™ ECG unit is a bioamplifier*

# INTRODUCTION

Assessment of cardiac physiology is integral for studies that:

- Examine the effects of therapeutic interventions
- Experimentally induce pathology
- Describe phenotypes linked to gene and molecular variants.

The heart generates its own electrical signal, which can be measured using the electrocardiogram (ECG). The ECG detects the extracellular voltage change that is produced as heart cells depolarize prior to contraction. As the signal moves through the heart in succession during each heartbeat, data collected from an ECG can inform on heart rate, ahrrythmia, chamber defects, channel pathologies and more. The **CardioPhys™ ECG** system is a highly versatile tool for measuring ECG of a wide spectra of animals – as small as a zebrafish embryo to animals as large as an alligator.

The **CardioPhys™ ECG** unit is a bioamplifier that is designed to amplify the sum of electrical potentials from cardiac muscle, and other extracellular signals. The signal produced from cardiac recordings tracks the depolarization and repolarization of the

cardiomyocytes as they contract in succession to pump blood through the heart. The amplifier is powered by two 9 V batteries to minimize electrical noise, which could interfere with or mask the voltage signal from the heart. The **CardioPhys™ ECG** unit receives its input signal from a head stage connected to two electrodes, then sends the signal output to a data acquisition system as a voltage signal. The electrodes are attached to the animal across the area where an electrical field is generated by the heart. An additional third electrode serves as a ground, which can be either attached to the animal or immersed within a conductive medium surrounding the animal. When the electrodes are attached to the animal, the ground wire serves to complete two circuits, one from each electrode to the common ground electrode.

The **CardioPhys™ ECG** unit has high pass and low pass filters.

- The high pass allows only frequencies above a specified value to "pass," and eliminates any frequencies below that set value.
- The low pass filter allows frequencies below the specified value to pass and eliminates any frequencies above that set value.

A position dial can adjust the zero, in case of signal drift. The amplification of the signal can be increased from 100X, to 1,000X to 10,000X the incoming signal.

The **CardioPhys™ ECG** unit also includes electronics within the system (both the amplifier and the head stage) that are maximized for low noise recording of very small signals using either glass or metal microelectrodes. The **CardioPhys™ ECG** unit is versatile enough to measure extracellular voltage signals from animals as small as a hatched zebrafish embryo to animals as large as an alligator (or elephant) to record electrical potentials at the cell or tissue level.

## Features

- Input Mode: AC
- Differential Input Configuration
- Gain Range: 100-10,000 (AC)
- High Pass/Low Pass Filters
- Offset Position Control
- Current Generator
- Remote Active Headstage
- Power supply: 9 V alkaline batteries (2)

## The Electrocardiogram

The **CardioPhys™ ECG** is ideally suited to measure electrical potentials from cardiac muscle. The signal produced from cardiac recordings tracks the depolarization and repolarization of the cardiomyocytes as they contract in succession to pump blood through the heart. The result is the electrocardiogram (ECG). Information captured from an ECG can be used to characterize phenotypes that cannot otherwise be seen. ECG data can also be diagnostic of cardiovascular effects caused by stressors such as toxicants or other environmental variables. Unlike an action potential, the ECG is the sum of all electrical activity generated over time from all of the action potentials occurring in the heart. The ECG signal deflects up or down, depending on the flow of charge in the extracellular spaces.

## *Principal*

The intracellular voltage at the edge of the cell membrane is negative while the voltage outside the cell is slightly positive. When the cells depolarize, polarity inverts, such that inside the cell membrane becomes positive, and outside the cell becomes negative.

The cardiac cells in the heart chambers depolarize quickly in a successive wave as the signal spreads from cell to cell through gap junctions, causing the cardiomyocytes (heart muscle cells) to contract. The **CardioPhys™ ECG** records of the depolarization events as the signal spreads from cell-to-cell across the heart muscle. It keeps a constant watch on the extracellular environment, so that it can sense the presence of negative ions while they move across the extracellular space of the heart tissue, as the cells depolarize, then repolarize, one after the other. A cell may depolarize from –70 mV to +130 mV, which is quite a difference in the extracellular environment. However, the **CardioPhys™ ECG** will record a much reduced change in voltage (i.e. 1 mV), since the signal dissipates as it moves from the heart to the surface of the body where the electrodes are positioned. Because of this reduction in voltage, we use very sensitive electrodes and recording equipment to capture the signal of the heart. Once electrodes are positioned, as the voltage signal from the heart flows towards the positive electrode, the ECG signal will deflect up. When the voltage signal from the heart flows towards the negative electrode, the ECG signal will deflect down.

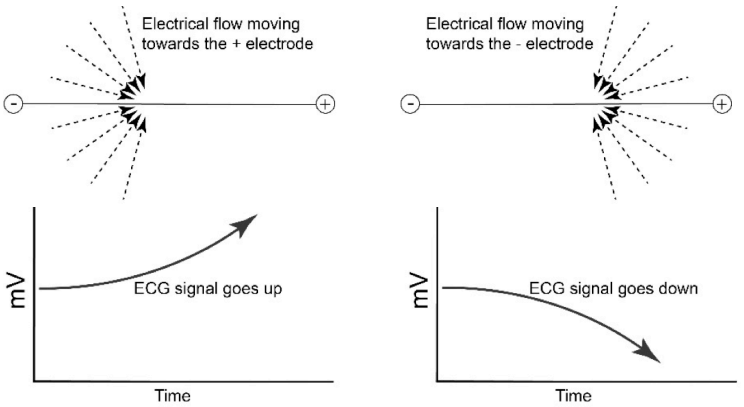


*Fig. 2—Deflection of ECG signal. The ECG signal will go up when depolarization wave moves towards the cathode. ECG signal will go down when depolarization wave moves towards the anode. If the depolarization wave moves perpendicular to the orientation of the electrodes, the signal stays flat.*

As the atrium and ventricle move through one beat cycle, each chamber depolarizes before a contraction, then repolarizes after the contraction. If the electrodes are positioned correctly on an organism with a fully chamberized heart, the ECG waveform will retain waves, segments and intervals that can be interpreted to describe the activity of the heart. Waves are any part of the signal that goes up or down, depicting the depolarization and repolarization activity respectively. A wave of depolarization traveling towards the positive electrode will cause a rise in the ECG signal, and when that same tissue repolarizes in the opposite direction, there is a decrease in the ECG signal. The waves of the ECG are

the P, Q, R, S and T waves (Fig. 3). Segments are sections between waves where there is no change in signal. Intervals of the ECG are combinations of waves and segments that relate to important events during the cardiac cycle.

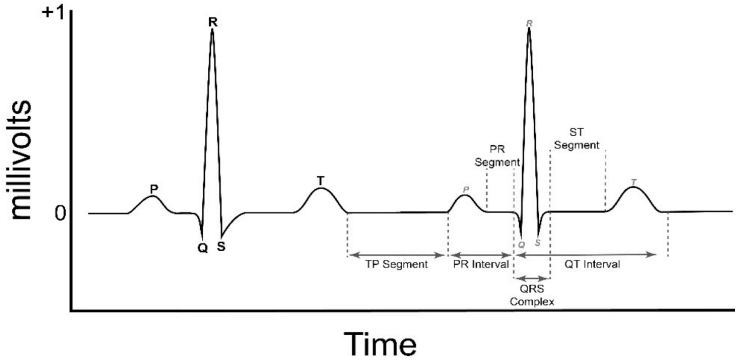


*Fig. 3—Waves, segments and intervals in the ECG signal. The P, Q, R, S and T waves represent events that deflect the signal up or down, depending on the direction of the voltage with respect to the electrode configuration. Segments are time periods between waves. Intervals are events of interest, which may include waves and segments.*

Once electrodes are positioned across a fully chamberized heart, the ECG will record the signal as the depolarization/repolarization events spread across the heart. As the depolarization spreads along the atrium, the signal is deflected upwards to create the P wave. Each cell in the atrium contracts just after the signal passes. The QRS complex is formed by the strong signal of depolarization in the ventricle. During the time the QRS complex is formed, the atrium is depolarizing, however, this event is lost in the ECG signal, due to the much stronger signal of the ventricle. As the ventricle is becoming depolarized, it is also contracting as the signal spreads. The repolarization of the ventricle is seen as the T wave.

## *Waves, Intervals and Segments*

- **P wave** – Depolarization of the atrium
- QRS complex ("R wave") – Depolarization of the ventricle (and repolarization of the atrium)
- **T Wave** – Repolarization of the ventricle
- **PR interval** is the distance (time) from the beginning of the P wave to the beginning of the Q wave. This interval is not called the PQ interval, because it is not uncommon that the Q wave is not visible on an ECG from a healthy organism. As such, the name PR interval stands. The PR interval is the time from the beginning of atrial depolarization plus the beginning of atrial depolarization plus the PR segment (see next).
- **PR segment** is the time lag between the completion of depolarization of the atrium (the end of the P wave) and the beginning of the depolarization of the ventricle. An increase in PR interval due to increased PR segment indicates a delay in conduction

from the atrium to ventricle. Any elongation of PR can indicate AV block, where conduction between the atrium and ventricle is decreased. In humans PR elongation is referred to as first degree atrioventricular block and suggests that the AV node is damaged or otherwise malfunctioning. In other organisms, such as fish, there is not a distinct AV node, though a ring of cells surrounding the area between the atrium and ventricle are suspected to serve the same purpose of delaying the ventricle activity to keep the heart pumping synchronously.

- **QT interval** is the time between the beginning of the Q wave (or R wave if a Q wave is not present) to the end of the T wave. This represents the entire time of depolarization and repolarization of the ventricle, so it is often linked to action potential duration in the ventricle. QT prolongation can be a sign of ischemia and can lead to lethal arrhythmia in humans. However, a shortened QT interval indicates a faster repolarization and shorter action potential duration. This shortened QT interval is often associated with fibrillation. Since the QT interval includes repolarization events in both atrium and ventricle, QT prolongation may be indicative of insufficient repolarization in either or both tissues. Indeed, a number of drugs can cause QT prolongation due to effects on repolarization. Additionally, QT prolongation can be congenital (Congenital Long QT Syndrome) and is linked to a number of mutations mostly associated with ion channels[1].
- **ST segment** is the time between the completion of ventricle depolarization and beginning of ventricle repolarization. As such this segment of time represents the ventricular action potential plateau, which is characteristic of cardiomyocytes. If there is a prolonged ST segment, an increased action potential plateau could be due to channel pathologies involving the $Na^{+}$ $Ca^{2+}$ exchanger and $Ca^{2+}$ channels chiefly responsible for maintaining the plateau.
    - The **T Wave** itself is not part of an interval, however, alteration of the T wave can be pathological when the repolarization of the ventricle is affected. A reduction in duration of the T wave, alteration in shape (i.e. inversion, flattening) can indicate ischemia.
- **TP segment** is the time between cardiac cycles.
- **RR interval** is often measured as the time between heart cycles. Alteration of the RR interval can be indicative of arrhythmias and can be averaged for obtaining the heart rate.

**NOTE**: Each event can be a segment of interest. For example, the duration of the QRS complex, the P wave, the T wave can be of interest.

**NOTE**: During a recording, waves may be misrepresented if the electrode configuration is not optimal. Likewise, if the morphology of the heart is different between samples, the shape of the waves will be different. This makes direct comparison between signals from different developmental stages is difficult.

## Parts List

- (1) **CardioPhys™ ECG** Unit
- (1) **ECG-PROBE CardioPhys™** probe with low-noise, active headstage
- (1) **300726 CardioPhys™** reference electrode assembly
- (1) **MD4R** or **MD4L** Dual Tool Holder Micromanipulator (**CARDIOPHYS-PRO** version only)
- Startup Kit
  - (1) **2033** Black Insulated Mini Banana Plug for metal electrode connection
  - (1) **2034** Red Insulated Mini Banana Plug for metal electrode connection
  - (1) **5470** 0.031" Jack for metal electrode connection
  - (2) **MEH7Wxx** Microelectrode Holder for glass eletrode [Choose size 1.0 mm (**MEH7W10**), 1.2 mm (**MEH7W12**), 1.5 mm (**MEH7W15**) or 2.0 mm (**MEH7W20**)]
- (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 27 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 27 of this manual.

## Cautions

Since there are a number of small parts in the package, WPI suggests setting up a designated storage space for upkeep and care. Always work with clean hands and maintain all parts by keeping them clean and protected from damage.

**NOTE**: Working with electrolyte solutions will certainly leave salt residue on all items. The salt will not only corrode metals, it will also interfere with reliable recording of low voltage physiological data.

# INSTRUMENT DESCRIPTION


*Fig. 4—CardioPhys™ ECG unit*

**Low Pass Filter** rotary switch determines the lower cut off frequency.

**NOTE**: At the 0.1 Hz setting, the **CardioPhys™ ECG** may take a long time to recover if the input wire is inadvertently disconnected or if the input is exposed to an excessively large electrical signal. This is typical of AC amplifiers set at low frequency filter settings. For fast recovery from signal overload, operate at the highest frequency setting of the low frequency filter consistent with the application. Generally, slow signals such as ECG and EEG require the 0.1 Hz setting.

**High Pass Filter** selector switch affects the **CardioPhys™ ECG** response speed. The 10k Hz setting (widest band setting) enables the **CardioPhys™ ECG** to respond most quickly to rapid signals. However, noise is more prominent in wideband operation. Therefore, use the lowest HIGH FILTER setting consistent with minimal degradation of the required bio-signal. For ECG and EEG 100 Hz will be adequate.

**Position** knob applies an offset value that can move the baseline output reading up or down as much as 250 mV.

**Gain** rotary switch selects the magnification of the amplifier. The amount of signal magnification can be set to 100x, 1,000x or 10,000x.

**Battery Test** button tests the battery. When pressed a short, audible tone should sound, indicating that the 9 V batteries are good.

**Power** switch turns the amplifier on (I) and off (O).

**Output** port is used to connect the **CardioPhys™ ECG** with a recorder or oscilloscope.

**Probe Input** port is used to connect the probe.

*Fig. 5—(Left) CardioPhys™ ECG probe with head stage*
*Fig. 6—(Right) CardioPhys™ ECG reference electrode*

The probe head stage is epoxy encapsulated within a miniature gold plated metal case. The use of a probe, close to a recording site, minimizes the shunt capacity normally associated with long wire leads from the electrode to the amplifier.

**CAUTION**: The probe head stage is sensitive to static discharge and can be damaged by a high voltage shock.

# OPERATING INSTRUCTIONS

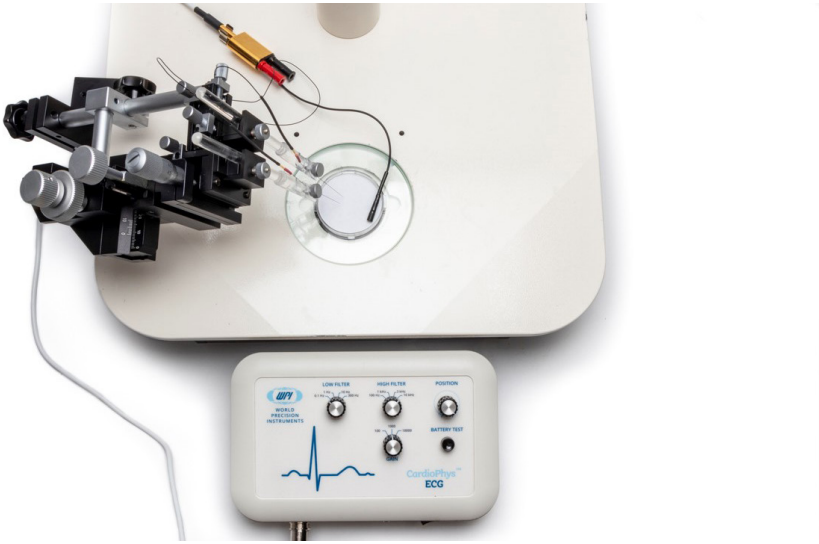


*Fig. 7—This is a typical setup of the CardioPhys™ ECG system.*

## Preparing Accessories

The following accessories are not included with your system, but generally used with the **CardioPhys™ ECG**.

**Glass or metal electrodes** – A wide variety of metal electrodes may also be utilized and might be ideal for your experiments. Larger animals may be best recorded using customized electrodes. Contact customer support to provide ideal electrode solutions for your model organism.

Tungsten metal electrodes are ideal for small animals (and small signals). Tungsten electrodes come in a variety of shapes and configurations. For larger animals, a stainless steel needle electrode will be adequate. Be aware that if a metal electrode comes in contact with a media bath, the signal from the animal will be diminished. Be sure to insert or otherwise protect the electrode from a watery media to ensure that the connection to the animal is better than the connection to the surrounding media. Inserting a metal electrode may require a minor surgical procedure to ensure the electrode stays in place if the organism is moving. However, movement artifacts can be troublesome, so appropriate light anesthesia or restraint is suggested. Contact customer service for suggestions.

Glass electrodes are ideal for small aquatic organisms. The glass creates a seal around the skin of the organism so that a highly conductive electrolyte is in contact with the organism, but not the conductive media bath. If you are recording from an aquatic organism with scales, the scales should be removed from the recording site to create a smooth surface

for the glass electrode to contact the organism. The ground sits in the media bath (or can be inserted or attached to larger organisms). Glass pipettes are fragile and must be handled with care because the thin glass at the small diameter opening is easily breakable. However, glass electrodes are sensitive and cost-effective.

**Glass Pipettes** – Glass electrodes are pulled glass pipettes with 1.0 mm O.D. with 2 µm tip are part #**TIP2TW1**. Once filled with 3M KCl these glass pipettes are now effective electrodes. A 3X Danieau solution (physiological saline for zebrafish) will produce electrode resistance between 2-6 MΩ.[2] The electrodes are connected to the system using electrode holders.

**TIP**: Don't let the tip of the electrode dry out. Salt buildup from evaporation can cause the fragile tip of the glass electrode to break and can cause poor signal readings. Make an electrode storage dish by filling a small dish with electrolyte bath (see below). This electrode storage dish will ONLY be used to keep the electrodes from drying out when not in use.

**Electrolyte Filing Solution** – For glass pipette electrodes, the most commonly used electrolyte solution is 3M KCl. NaCl can also be used. A 3 M solution is not necessary for ECG recordings if you do not have KCl on hand. Phosphate-buffered saline (PBS) and other physiological saline solutions (Ringer's, E3 medium, Danieau Solution, etc.) can also be used in lieu of KCl, since the conductivity can be similar if the concentration is increased. Increased electrolyte concentration decreases the resistance to current flow, so higher concentrations will lower the resistance. Notably, a 1X solution of PBS may be adequate as an electrode filling solution in most situations, though increasing to a 3X solution may provide enhanced recording. For media bath and holding during experiments, a 1X physiological saline appropriate for the species and/or developmental stage might be preferred. Descriptions below will use a 3M KCl solution, due to its common use and consistent results.

**Electrolyte Bath** – A conductive media is often used for aquatic organisms. If the organism is within this bath, the ground can be submerged in the media. Appropriate physiological saline for the species or other appropriate conductive solution will be adequate. Phosphate buffered saline, E3 medium, Danieau Solution and sea water solutions are commonly used.

**NOTE**: The electrode solution should be several times more conductive than the electrolyte bath. For example, if you are using 1X PBS, fill your glass electrode with 3X PBS, 3M KCl, etc.

**Electrode Holders** – For glass electrodes, an electrode holder with a chloridized silver wire must be used. To make a glass electrode, a glass pipette is first filled with conductive electrolyte solution (see below), then the glass pipette must be attached to an electrode holder. The electrode holder has an integrated silver wire that will be inserted into the glass pipette (see below). The chloridized silver wire provides a large surface area for enhanced conduction of signal.

**NOTE**: Silver wires do not come pre-chloridized since they must be rechloridized regularly as part of electrode holder maintenance.

To chloridize a new silver wire on an electrode holder, or to re-chloridize a used wire (must

be done at least daily), soak the wire in unscented household bleach. This can be done in a 50 mL tube, or small dish. After approximately 20-30 minutes the shiny silver wire will turn dark grey or become otherwise darker when chloridization is complete. A range of colors have been reported following chloridization, though dark grey is most common. Once chloridized, rinse the electrode holder and the wire well in deionized water to remove residual bleach.

**TIP**: Keep extra chloridized electrode holders on hand. Since a silver wire loses its chloridized layer over time, this could interrupt an experiment. If extra chloridized electrode holders are on hand, simply swap out the electrode holder.

**TIP**: Make sure you have extra O-rings (one extra is provided). Over time the bleach will degrade the rubber O-ring. You could alternatively remove the rubber O-ring each time you re-chloridize to get a longer life from your O-rings.

**Glass Electrode Assembly** – To complete the assembly of the glass electrode:

1. Fill a glass pipette with conductive electrolyte solution (see below).
2. Insert the chlorided wire into the pipette as you attach the glass pipette to an electrode holder.
3. As the electrolyte-filled pipette is inserted into the electrode holder, it will pass through a rubber O-ring. Once the end of the glass passes through the O-ring, it can be secured by tightening the collar of the electrode holder.

**CAUTION**: Do not push the pipette to the extreme back of the electrode holder. The back edge of the glass could damage the connection between the silver wire and the electrode holder connection.

**MicroFil and syringe** – If you are using glass electrodes, a **MicroFil** is commonly used to fill the glass electrode with electrolyte. The MicroFil is a small diameter blunt tube that attaches to any syringe. Fill the syringe with electrolyte, insert the MicroFil into the glass pipette and advance towards the tip and gently fill with solution. Be sure to remove all air bubbles.

**CAUTION**: Excessive pressure exerted from the syringe can break the delicate tip of the glass pipette.

**E210 Electrode storage containers –** It is convenient to hold electrodes in a secure location. This prevents permanent damage to the tip of the electrode which can easily occur with even the slightest touch, especially with glass electrodes. Electrodes can be as small as a few microns and are difficult to replace during an experiment.

**Micromanipulators** – For small organisms, micromanipulators are needed to move the electrodes and to maintain the position for extended periods of time. Several configurations of electrode placement may be necessary during troubleshooting. Individual variation may also cause different configurations to be necessary between organisms. During early development this variation may be exacerbated, while organisms

develop along slightly different developmental trajectories. As such, electrode placement may be different between organisms of the same early developmental time point. To best account for this variation, two micromanipulators are necessary to provide independent movement of each electrode.

**TIP**: A third micromanipulator may be used to hold the ground wire. For small fish, the ground wire is often set in the bath, since the bath is conductive. The ground should be secured in place to prevent movement artifacts in the recorded signal. If an organism is above water or terrestrial, the ground should be attached to the organism. A needle electrode can be inserted subdermally or affixed with a small clamp. Additionally, a surface electrode may be effective as the ground electrode. Regardless, having the ground secured in place is often desirable.

**Head Stage Holder** – The head stage is delicate and crucial for collecting a clean signal, so it should be protected and held in place. Affixing the head stage to a micromanipulator provides an ideal and secure positioning between the organism and the **CardioPhys™ ECG** unit.

**NOTE**: Keep the head stage clean and free of salts. Salts on the head stage (or other pieces of the workstation) can cause signal artifacts. Maintain a clean and shiny head stage for best practice.

**Microscope** – Your workstation will certainly be configured to personal preference, and your method of magnification will be based on your needs and preferences. There are a multitude of options available for magnification. A surgical microscope on a swing arm is ideal for use with small animals. A metal base provides a surface to attach magnetic stands for micromanipulators. Micromanipulators will need to be moved and repositioned, so a magnetic stand is convenient and advantageous for providing a solid base for anchoring the micromanipulator, and for easily moving the micromanipulator when needed. For small animals, a solid base of operation is imperative. Also for small animals, a microscope with a long working distance objective (>70mm) is strongly suggested to allow flexibility in in positioning and angling the electrode. If the objective has a shorter working distance the electrodes must be angled accordingly to fit between the microscope and animal, reducing the flexibility in electrode manipulation and placement. A longer working distance allows the objective to be further from the specimen, such that the electrodes can be positioned at steeper angles when necessary, but still can be angled lower, when needed. Other magnification methods may be adequate such as loupes, or industrial magnifiers. Digital handheld microscopes can be mounted and moved easily and may be ideal in some scenarios. The most important feature is that the magnification should not be in the way of the preparation and micromanipulator. Several microscopes have a large magnetic stage to allow you to equip your workstation on the microscope, as well.

**Lighting** – Good lighting is essential. For microscopy, an LED gooseneck light system is suggested. Lights may need to be turned off and on periodically to investigate the influence on electrical interference of the bio-signal. If using a halogen light, be sure to turn down the intensity before switching it off or on to preserve the bulb life. An additional lamp is also convenient when filling pipettes and for extra light.

**Table** – A sturdy table is important. Minute table movements can produce movement artifacts in the signal. However, an anti-vibration table is not likely needed. A metal table top is suggested to accommodate magnetic stands.

**Animal holding accessories** will vary depending on the organism. A dissection tray is often ideal for larger organisms. For small fish models, petri dishes filled with agarose (>2%) are commonly used. **Z-MOLDS** are pressed into the melted agarose to create wells and troughs for holding the fish. Customization of **Z-MOLDS** configuration is easy and effective with a small #11 scalpel blade. Keep agarose sealed and humidified and these molds will be reusable multiple times.

Paraffin is also used as an alternative to agarose. Paraffin provides a more solid substrate and can be customized by carving wells into the wax. To use **Z-MOLDS** with paraffin, we suggest using paraplast embedding media, which will allow the **Z-MOLDS** to be easily removed when chilled. It is not recommended to use a mold release spray, since many are toxic.

**NOTE**: Small insect pins and high gauge needles are effective at holding fish in place within molds. Many species of cactus spines have barbs that hold well in agarose. View several species cactus spines under magnification to see a wide range of shapes and configurations including hooks and angles for securing small specimens.

Holding methods are also chosen due to personal preference and may be configured to incorporate other aspects of your workstation. Regardless, the animal must be held securely, since the electrodes must make firm contact with the organism and movement can cause artifacts in the signal.

**NOTE**: Organisms should be sedated or otherwise restrained. Movement artifacts will appear as low frequency signals that can obscure ECG data. Breathing in terrestrial organisms, opercular movement during gill ventilation, and spinal reflexes are examples. These signals can be filtered digitally from the signal and are of little consequence if anesthesia is not ideal for the experimental protocol.

**Surgical equipment** – A variety of forceps are commonly used for various tasks such as moving microelectrodes, organisms or other small items. Fine scissors, probes and scalpels may also be useful. Other surgical tools may also be needed to manipulate organisms or to modify animal holding. As mentioned above, modifications such as small slits can be made in agarose and insect pins work well in paraffin. Dissection pins or other standard methods are used for larger animals.

**Containers** – A squirt bottle with deionized water should be on hand for cleaning. Constantly police the inevitable buildup of salt to prevent erroneous readings. Every surface should be kept clean of salt including wires, table, knobs, connectors, head stage, etc. Extra containers to hold electrolyte or to wash equipment is also suggested. Keep a lint free cloth dampened with deionized water on hand to stay ahead of this potential problem, as buildup of even a minute amount of salt will cause problems in recordings. Extra beakers and dishes may be useful for managing liquids or organisms.

**Sharps container** – When dealing with glass or metal microelectrodes, keep a small sharps container nearby for disposal of these and other sharp objects.

**Computer and data acquisition system** – A computer and data acquisition system (e.g. Lab-Trax) are needed to view and record the data. Sampling rates >400 samples/second may be desired to obtain clean data for analysis. As such, a computer with adequate storage space and processing power will be needed. In most cases, a typical office grade desktop or laptop computer will suffice. For higher sampling rates (>1,000 kHz (samples per second)) a larger hard drive, faster processing speed and more RAM may be needed.

**NOTE**: It is important that you be familiar with the data acquisition system to be able to record and analyze data. Read completely all manuals and tutorials available prior to experimentation. Several commercial data acquisition suites are available. Contact customer service for help in choosing a data acquisition system. If you already have a working data acquisition system and software suite, the voltage input will certainly receive the signal from the **CardioPhys™ ECG** unit.

## Setting Up Your Workstation

Once you have familiarized yourself with the parts, and the elements are all ready, it is time to set up your workstation. Here, we describe an ideal workstation for small fish (<7 mm) using glass electrodes. The **CardioPhys™ ECG** unit is well suited for recording ECGs in numerous other model organisms, including mammals. Larger fish and other model organisms require other considerations in electrode design and placement, so contact technical support for assistance in customizing your workstation and choosing the optimal electrodes for your research. This procedure is an example of how a researcher can use the **CardioPhys™ ECG** system.

**NOTE**: The sensitive electronics in the **CardioPhys™ ECG** unit are also optimal for recording nerve and muscle activity.

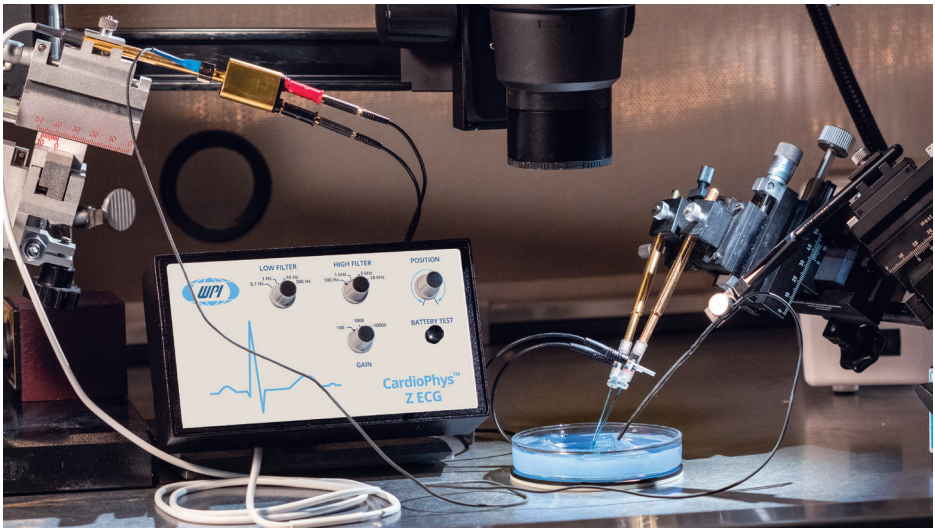


*Fig. 8—ECG monitoring using WPI CardioPhys™ ECG system.*

1. To set up your workstation, attach each electrode holder to a micromanipulator.

   **TIP**: A left and right-handed micromanipulator facing opposite directions work well together. Or, two micromanipulators can be placed side-by-side, if desired. A dual electrode micromanipulator is also an excellent choice for efficient placement of electrodes and workstation design.

2. Next secure the head stage to a third micromanipulator or to an otherwise secure location near the electrodes.

   **NOTE**: A single electrode configuration is often desirable to simplify your experiments, especially if the organism is particularly small (e.g. fish larvae). To configure a single electrode recording scenario, prepare one electrode holder by connecting it to the red (non-inverting) plug on the Head Stage. Connect the black (inverting) plug on the head stage to the ground clamp using a short jumper wire. Now you can use only the red side of the head stage, connected to an electrode (and the ground) to make your measurement. This configuration is reasonable and quite effective at measuring electrophysiological signals. However, the benefits of signal noise rejection by using a differential amplifier are now not as great. Even so, this configuration may be perfectly adequate to achieve high resolution ECG data from your experimental organism by using digital filters.

3. Fill glass pipettes with electrode filling solution (i.e. 3 M KCl, etc.).

   **TIP**: Add a little methylene blue to color the electrode filling solution to make it easier to see the glass electrode.[3]

4. Attach the electrolyte-filled glass pipettes to the electrode holders.

5. Connect the electrode holders to the head stage using the wires provided.

6. Using the micromanipulators, position the electrode tips so that they are next to each other in the center of the electrode storage dish.

7. Adjust the position of the micromanipulators to accommodate the microscope.

8. Use the microscope to:
   - Ensure the electrodes are immersed in the liquid in the petri dish.
   - Adjust the position of the electrodes so that each electrode can cover the same 1 cm diameter area. This dual coverage ensures that electrodes can be repositioned on any area of a small fish as needed, without moving the base of the micromanipulator during experimentation.

9. Connect the ground to the head stage using the clamp. The clamp snaps on to the post of the head stage to complete the ground connection. The ground wire for a media bath should have a silver pellet connected to the wire that is immersed in the media. To avoid unnecessary movement artifacts from a shifting ground wire, firmly secure the ground electrode to a single location in the media bath. Use a clamp, micromanipulator or some other method to ensure that the ground is secure in the media filled dish.

**NOTE**: A ground can be attached to the fish by using a small needle electrode as the ground wire and inserting it in the musculature of the organism. Surface electrodes and clamps can be used on larger animals. Contact customer service for additional options for larger or unique configurations.

10. Connect the head stage to the **CardioPhys™ ECG** unit using the 5 pin round plug.
11. Connect the **CardioPhys™ ECG** unit to data acquisition system using the BNC cable to the appropriate input on the data acquisition system. If there are multiple inputs, select the first input (#1).

    **TIP**: Make sure you have more than double the glass electrodes you think you need. They get broken with the slightest touch. Even a zebrafish larvae or salt crystals can damage the electrode tip.

## Connecting to Animal - Data Acquisition and Analysis

A number of data acquisition software tools are available to capture, record and analyze voltage data. Commercial data acquisition software packages have a number of features in common that will help you best capture the voltage signal coming from the **CardioPhys™ ECG** unit, to filter any unwanted electrical noise picked up from the environment, and to interpret the physiological signal. Lab-Trax is a popular software suite and is supported by WPI. However, the following steps for data acquisition and analysis will apply to any of the major data acquisition software suites.

### *Before Connecting Electrodes to an Animal*

1. Turn on the data acquisition system and computer and launch the data acquisition software.
2. Turn on **CardioPhys™ ECG** unit. Since all the wires are connected and the electrodes are in the media, there should be an incoming signal on input 1

### *Setting Up Incoming Data Stream*

When your are first starting out, it is a good idea to pre-adjust the settings in the data acquisition software before attempting a recording.

1. Set the sampling rate for Input 1 to 1000 samples/second to start. An ECG is effectively sampled at 400 samples per second. However, a higher sampling rate may be helpful in sorting proper filters and detecting signal nuances in the ECG.
2. Several high frequency signals will likely be apparent creating an oscillating or otherwise messy signal. This interference can be removed using filters. Familiarize yourself with the data acquisition software manual and online help.
    - The analog low filter on the **CardioPhys™** unit can be used to eliminate much of this noise, by only allowing signals below a chosen frequency to pass through to the data acquisition system. However, filtering the raw signal can never be reversed, so WPI suggests that these analog filters be used sparingly, in lieu of digital filters present in the software.

- On the **CardioPhys™ ECG**, a 0.1-1 Hz Low Filter and a 0.1 kHz High Filter setting is a good start with the gain at 100X or more likely, 1000X.

  **TIP**: Add a second channel to your ECG data file. The first channel will be the input channel where the raw data is directly displayed using conservative values for any analog (hardware) filters (i.e. 0.1–1 Hz low filter and 0.1 kHz high filter). The second channel in the software is just a duplicate of the first channel. You can use this second channel to apply digital filters present in the software to clean up the signal by removing other frequencies. If filtering becomes difficult, you can also duplicate input channel 1 to create additional channels that mirror the input channel to experiment with different filters until you find the correct combination of filter, sampling rate, range, etc. that best suits your data stream.

- Mains interference is common in laboratories. It is generated by all of the electrical devices in the lab including freezers, centrifuges, lights, fans, etc.
  Some data acquisition devices have a hardware mains filter and other optional hardware filters within the data acquisition device. As stated above, once you remove signal data using hardware (analog) filters, you cannot go back to the raw signal once you record. So, again, be conservative if you are unsure of the use of the analog filters.
  It is NOT recommended to use any mains filter or other analog filters that may be present in the data acquisition device. It is preferable to retain the raw signal with the most conservative values chosen on the **CardioPhys™ ECG** unit, then to use digital filters within the software to further clean up the signal.

  **IMPORTANT NOTE**: Digital filters can be applied, altered and removed during and after data recording. Once an analog/hardware filter is used, it can never be removed, because these analog/hardware filters permanently alter the signal, and therefore the data. This can result in permanent data loss. Use digital filters whenever possible to fine tune the signal.

  **NOTE**: The **CardioPhys™ ECG** has superior inherent noise rejection. However, interference can still occur as artifacts of the environment. A Faraday cage and proper grounding will minimize noise interference. Contact technical support for additional suggestions to minimize noise.

- You may be able to use a spectrum view function to analyze the incoming frequencies in real time. This function is available in most software. Once interfering frequencies are identified, the appropriate filter can be more easily chosen and applied.
  For ECG data, analysis commonly only requires a low pass filter to remove the high frequency noise that oscillates along the trace line.
  However, a band pass filter is a combination of a low and high pass filter. This can remove both the high frequency mains signal, and very low frequency muscle twitches to obtain a clean signal.

  **NOTE**: When you first acquire a signal from the organism (below) selecting the appropriate sampling range (mV) and sampling rate in the data acquisition software settings can also reduce the appearance of noise in the signal.

3. Select the appropriate sampling range (in mV). All data acquisition software suites have an autoscale option that will pick the appropriate range of data collection. Once you remove a bit of noise from the incoming signal, you can pre-select the sampling range. You want to avoid, for example, sampling at a 10 V range from 0, if you have a 0.2 V max signal. IF you are sampling at a large range, you could just zoom in and see the signal, however, you will be losing significant resolution of your signal. If your signal is 0.2 V max, you would choose something like 0.5 V. Your signal will be in the mV range.

4. Additionally, set your gain to 100 or 1000X. This will gently amplify your signal to a serviceable amplitude in the low mV range.

   **NOTE**: It is not always necessary to record data during setup of the system. However, it is an effective learning tool to record data from your first few set ups while you are getting used to the equipment. All data acquisition software suites allow notes to be placed into the data stream with a time stamp. You can make comments and notes of what was done over time to record your methodology within the data stream. This can be an effective trouble shooting tool and can help you remember what was done.

## *Preparing Your Organism*

1. Prepare your fish such that it is secured within a holding dish for measurement. This is often done at another prep station close to the recording station.

   **NOTE**: Having a separate animal prep station is advantageous, because electrode tips are commonly damaged while manipulating the fish. Some manipulation of the fish under measurement will be necessary, but minimizing the activity near the electrodes is preferable.

2. Utilize proper anesthesia to immobilize the fish. 100-300 mg/L MS-222 in media, buffered appropriately, is often used for larval fish. 100 mg/mL is sufficient for obtaining longer term measurements in larval zebrafish. Other species are more or less sensitive and concentration can be adjusted down for species, such as killifish or up for larger or more resistant fish.

3. Physically secure the fish in the measurement dish. Even though the fish is anesthetized, the fish should be physically secured to provide a stable point of contact for the electrode(s).

4. Utilize temperature control, as necessary. Several thermometers have a voltage output to connect to the data acquisition system to continuously monitor temperature.

   **IMPORTANT NOTE**: In poikilotherms heart rate is significantly affected by temperature. As such, temperature should be monitored.

## *Placing Electrodes on the Organism*

1. Once the fish is secured in the measurement dish, place the fish beneath the electrodes by moving the measurement dish.

   **TIP**: It is a good idea to secure the measurement dish to the table. Small movement of the measurement dish can cause electrode damage.

2. Reposition the electrodes over the fish, re-secure the ground in the media bath, and adjust the level of the media bath as needed.

   **NOTE**: Measurement can be done in a very small volume by utilizing the troughs and wells of the **Z-MOLDS** or customizing paraffin holding chambers. Remember to situate the ground electrode such that it is securely held in place, especially when working in small volumes.

   **NOTE**: A larval fish typically does not need to be completely submerged, though some liquid is necessary to complete the circuit through the fish to the ground. Alternately, a small metal electrode can be inserted in the tail or musculature of the fish in lieu of a submerged ground.

3. Prepare to place electrodes on the fish across the heart by pre-positioning the electrodes on either side of the heart.

4. Move the electrodes into position by adjusting the micromanipulator. Apply a slight pressure to the outside of the fish to create a seal between the glass edge of the electrode and the skin of the fish. Ideal placement of electrodes is with the two electrodes on either side of the heart – with one to the left and posterior to the atrium and the other to the right of bulbus arteriosis. This is not always perfect. Movement to the sides and near the heart may be best.

   **NOTE**: If you are using the single electrode configuration, the electrode is best placed near the junction of the atrium and ventricle.

   **NOTE**: It is not necessary to pierce the skin.

   **IMPORTANT**: There should be a seal between the glass and the fish so the electrode filling solution is only in contact with the skin, not the surrounding media.

   **TIP**: Don't worry about the difference between the black (inverting) and the red (non-inverting) electrodes. Just position the electrodes. Signal averaging and filtering may reveal that the signal is more serviceable than the first appearance. Even if the ground is placed so that the signal is inverted, this is not a problem, since the data acquisition software allows you to flip (invert) the signal with a click. So, it may be most effective to simply invert the signal in the software instead of moving wires or electrodes.

   **NOTE**: Electrode positioning is crucial to obtain a fully serviceable ECG. However, development of heart morphology will change the shape of the ECG. This is especially obvious as chamberization proceeds. So, as the heart changes during development, the characteristic ECG signal with PQRST waves also develops. So, for example, if the heart is not fully chamberized, there may be no distinction on the ECG such that there may be no PQRST waves. There may be only one wave for each beat. Indeed, an ECG signal from a larval zebrafish at hatch will appear significantly different than at one week, two weeks, etc. due to developmental changes in the cardiovascular system.

   **TIP**: It may be a beneficial to reduce the level of the media bath to allow the recording surface to be in the air, but this is not usually necessary. However, it is worth trying if there are difficulties seeing the tip or if you are unsure if there is good contact between the electrode and the fish.

   - As the fish develops and scales form, it becomes more difficult to make a clean

contact, so scales will need to be removed if they are present. A small micro scalpel is ideal, or a sharp pair of forceps or high gauge needle can also be effective at removing small scales. Additional difficulties become present when the opercula begin to envelope the area lateral to the heart, if electrodes are angled too low. Small iris scissors can be used to remove the cartilaginous aspect at the edges of the opercula, if they present a barrier to electrode positioning.

## *Adjusting the Settings*

1. View the signal on the recording software. Set the sampling rate to 400-1000 samples per second (kHz) and check that the gain is set to 100X. Turning the gain to 1000X or 10,000X may be necessary in some scenarios, but this will also amplify any noise. 100X should be good for most applications. On the **CardioPhys™ ECG** unit, set the Low Filter to 0.1-1 Hz and the High Filter to 0.1 kHz.

   Without altering incoming Input 1, duplicate the raw signal to a second channel, as mentioned above. Adjust the digital filter on the duplicated signal, leaving the raw signal unchanged. Pre-adjust the digital filters to better view the amplitude of the signal. A good starting point is a low pass digital filter between 25-50Hz. Once a signal is obtained that clocks heart rate, set the range for recording about 1/3 higher than the highest voltage seen. For example, if the voltage of the signal is between 0-150 mV, set the range at 200 mV or the next highest available setting. Again, do not set the range at a high range (e.g. 2 V), then zoom in to see the signal. It is important to set the range to closely bracket the voltage values you are recording, to take advantage of the resolution of the data acquisition system you are using.

2. There should now be some indication of heart rate within the signal. If available view the spectrum of the signal in the acquisition software. The heart rate of the fish will be around 120 bpm, which is approximately 2 Hz. Manually count the heart rate on the fish and the data acquisition system.

3. If the heart rate is not apparent, first adjust the digital filters to see any indication of a heart rate. It is normal to have no serviceable ECG signal at first.

4. Begin to filter the signal digitally with a low pass filter at 50 Hz and slowly move down at 5 Hz increments as you remove the higher frequency signals. The data line should start to get smoother.

   You might try a bandpass filter with a low of 1-2 and a high of 50 Hz. Adjust the high frequency down to remove some of the “wiggle” from the signal. Then adjust the low frequency up until you get an optimal signal.

   Again you may need to adjust the position of the electrodes.

   **TIP**: All data acquisition software suites have period/rate calculation apps within the software. Make yet another duplicate channel of the filtered signal. Use the rate function in the software to automatically calculate the time between beats. Modify the settings in the rate calculation app to choose the peak voltage or a voltage above a certain value to clock the heart rate (i.e. identify your R wave based on amplitude). Use the average function within the app to average multiple beats (i.e. 10-30 beats) to

obtain a live heart rate in beats per minute to compare to visually acquired heart rate.

5. Start recording the signal.

   **TIP**: In the beginning, record everything as you familiarize yourself with the system. Take advantage of the option of copying your raw data channel into several channels. This way you can apply different filter settings to the different channels to experiment with different settings. You can also create an analysis channel to calculate real time heart rate and many other variables within the signal.

6. You may have to adjust electrode position again once a signal is optimized.
7. Once a reading is stable, begin your experiment.

   **TIP**: Notes can be added within all data acquisition software systems available for measuring physiological signals

8. Once the experiment is complete, stop the recording in the software system and save the data file.

   **NOTE**: Data can be exported to a spreadsheet and analyzed offline or within the acquisition software to find all segment and interval durations by measuring the time between events. ECG analysis tools are often embedded in the software (such as the rate function) or available for purchase for real time or post acquisition analysis.

9. Turn off the **CardioPhys™ ECG** unit and disconnect the head stage wire and the data output wire.
10. Wipe the knobs and the surface of the unit with a damp cloth. Use water only, and do not soak the cloth. Use only a damp cloth or you risk damaging the equipment.
11. Retract the micromanipulators and remove the electrodes from the electrode holders.
12. Safely discard the glass electrodes.
13. Disassemble the electrode holders and wipe them down.
14. Move the organism to an appropriate location, and clean the holding area, as appropriate.
15. Wipe the knobs on the micromanipulators and carefully clean any salt residue.
16. Move the micromanipulators to a safe area so they do not get damaged.
17. Place the electrode holders in a bath of deionized water for a few minutes to dissolve any dried salt. Dry electrode holders prior to storage.
18. Wipe all the wires and any additional equipment that was near the electrolyte solutions with a damp cloth.
19. Clean all surfaces that were near the electrolyte solution(s).
20. Inspect the silver wires and rechloridize them in bleach, if necessary.
21. Move all equipment and accessories to their appropriate storage locations.

# INSTRUMENT MAINTENANCE

## Battery Test

The **CardioPhys™ ECG** is powered by two 9 V alkaline batteries. (Battery power offers lower internal noise level for electronic devices.) Test the batteries by pressing the BATTERY TEST push button. If a short tone is heard, the batteries are functional. If no tone sounds, the batteries must be replaced.

## Change the Batteries

Replace batteries annually or as required. Although any of the common 9 V transistor batteries will work well, alkaline cells are recommended for longer life.

1. Remove the four small, Phillips screws securing the rear panel of the instrument.
2. Remove the clips that secure the batteries.
3. Replace the two 9 V batteries with fresh ones.
4. Reinsert the battery clips.
5. Secure the rear panel with the screws.
6. Turn the POWER switch on and press the BATTERY TEST button. Listen for the audible signal.

# ACCESSORIES

| Part Number | Description |
|---|---|
| **MEH7W** | Microelectrode Holder, right angle |
| **M3301EH** | 2 mm Socket to 0.031 Socket adapter |
| **2033** | Black Insulated Mini-Banana Plug |
| **2034** | Red Insulated Mini-Banana Plug |
| **2101** | 9 V Alkaline Battery, each (2 required) |
| **ECG-PROBE** | ECG Probe |
| **300726** | Replacement Reference Electrode Assembly |

# OPTIONAL ACCESSORIES

Replacement parts can be ordered using the part numbers below. For additional accessories, refer to Accessories.

| Part Number | Description |
|---|---|
| **5052** | Steel Base Plate, 10 lbs. |
| **5484** | Steel Base Plate, 5 lbs. |
| **505198** | ECG module for Lab-Trax (Required for using Lab-Trax with the CardioPhys™ ECG unit) |
| **LAB-TRAX-4** | LabTrax 4-Channel Data Acquisition |
| **M3** | Tilting Base |
| **M4C** | Microscope Stage Adapter |
| **M10** | Magnetic Stand |
| **Variety** | Metal electrodes (choose from the low impedance electrodes) |

## SPECIFICATIONS

| | |
|---|---|
| Input Impedance | $10^{12}$ Ω, Common mode and differential |
| Input Leakage Current | 50 pA (Typical) |
| Gain | AC: 100X, 1000X, 10000X |
| Common Mode Rejection Ratio | 100 dB At 50/60 Hz |
| Input Capacitance | 20 pF |
| AC Mode Noise | 0.4 µv RMS (2 µV P-P) 0.1-100 Hz |
| AC Mode Noise | 2.6 µv RMS (10 µV P-P) 1 Hz-10 KHz |
| DC Bandwidth Filter Settings | |
| AC Mode | Low frequency, 0.1, 1, 10, 300 Hz |
| AC Mode | High frequency, 0.1, 1, 3, 10 KHz |
| Output Connectors | 3.5 mm Miniphone connector |
| Output Voltage Swing | ±8 V |
| Output Impedance | 470 Ω |
| Battery Test | Audible tone |
| Calibrator Signal | 10 Hz square wave |
| Position | Approximately 250 mV |
| Current Source DC Generator | 0 To ±50 µA, variable |
| External Command | Input voltage ±10 V commands |
| AC or DC Current Waveform | ±50 µa max. amplitude at 200 KΩ |
| Filter Type | Single-pole butterworth |
| Batteries | 2 X 9 V Alkaline (included) |
| Typical Battery Life | 500-700 hours continuous |
| Dimensions | 19.7 × 12.7 × 7.6 cm (7.75 × 5 × 3 in.) |
| Weight | 0.9 kg (2 lb.) |

## ENDNOTES


1 Leong, I. U. S., Skinner, J. R., Shelling, A. N. & Love, D. R. Zebrafish as a model for long QT syndrome: the evidence and the means of manipulating zebrafish gene expression. Acta Physiologica 199, 257-276, doi:10.1111/j.1748-1716.2010.02111.x (2010).

2 Forouhar, A. S. et al. Electrocardiographic characterization of embryonic zebrafish. Conference proceedings : ... Annual International Conference of the IEEE Engineering in Medicine and Biology Society. IEEE Engineering in Medicine and Biology Society. Annual Conference 5, 3615-3617, doi:10.1109/iembs.2004.1404016 (2004).

3 Dhillon, S. S. et al. Optimization of Embryonic and Larval ECG Measurement in Zebrafish for Quantifying the Effect of QT Prolonging Drugs. PLoS ONE 8, e60552 (2013).

## DECLARATION OF CONFORMITY

# WARRANTY

WPI (World Precision Instruments, Inc.) 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 one year* 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.*

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