IX-BIO4 Rodent Electrocardiogram

Equipment

1. IX-BIO4 Recorder
2. C-ISO-PN5 Needle Electrodes
3. C-USB Cable
4. Computer (Windows or Mac OS operating system)
5. C-PB-P1: Piggy-Back Male/Female Safety Pin Connector to Single Female Safety Pin Connector
6. Heater Controller and Temperature Monitoring System
   option a) SA-TCS-6, A-TCT-1 (Mouse)
   option b) SA-TCS-8, TM-BIO (Rat)

Supplies

1. Isoflurane inhalation anesthetics
2. Masking tape

Overview

The Electrocardiogram (ECG) is recorded and analyzed with the iWorx data acquisition and analysis system. Lead I and Lead II ECG is collected by connecting the needle electrodes to the four limbs of the animal. Lead III, aVL, aVF, and aVR can be calculated and analyzed in Labscribe. This system comes with the ECG Advanced Analysis Module, which allows for measurements of various intervals, amplitudes, and durations.

Downloading Labscribe

New Users: The equipment comes with a Temporary User Name and Password, so that you can immediately get started with your system by downloading and installing the software. You must register as a User with your own UN and PW. The one that comes with the system may be shared with your IT department as needed.

Important*: Labscribe must be installed under the administrator's account to enable data saving.*

Note*: iWorx has an open site license, permitting the software to be installed on any number of computers within the licensed site. This benefits researchers by enabling access from any location, allowing for off-site data analysis.*

1. Navigate to www.iworx.com on your computer.
2. In the toolbar at the top of the iWorx web page, select ‘Support’.
3. Select ‘Software Download’.

4. Select ‘Research’.

5. Select ‘Complete Installer’ for either Windows or Mac OS.

***Note**: On the LabScribe Software Download Page, there is a tutorial video titled “LabScribe101 – Basic Training” This video is a basic introduction to LabScribe to learn how to set-up, record and analyze your data.*

Recorder Setup

1. Place the recorder on the bench close to the computer.

2. Connect the USB cable to the recorder and the computer.

3. Open LabScribe software. A Device Status Dialog will pop up recognizing the devices.
    - From the Main window, select the **Settings** tab and then click **Load Group**.
    - Locate the **Research Settings** folder: C:\Program Files\iWorx\LabScribe\Research Settings
    - Open the IX-BIO folder which contains the settings group IXBIO.iwxgrp. Select this group and click Open.
    - Now that the settings group is loaded, select the **Settings** tab. Below “**Default Setting**” is a list of settings. Select the **Animal Heart** menu and then the **IX-BIO_SixLeadECG_Rodent** settings file from the list.
    - LabScribe will be configured by the **IX-BIO_SixLeadECG_Rodent** settings file, and a pdf setup document will open.

***Note**: Once the research settings are loaded, they will open the same every time LabScribe is launched. If you do not see the Research Settings folder in the LabScribe folder, reinstall LabScribe, making sure that the Research settings are installed.*

4. Insert the safety connector of the needle electrodes into the connectors on the IX-BIO4.
Note: The colors of the electrode wires may not match the colors on the ports of the IX-BIO4. The color of the needle electrodes does not matter. As long as the channels correspond to the correct limbs of the rodent, any wire can be used.

Equipment Setup for Single Channel

For a single channel of ECG you need the needle electrodes plugged into:

- Channel 1 positive port
- Channel 1 negative port
- Ground port

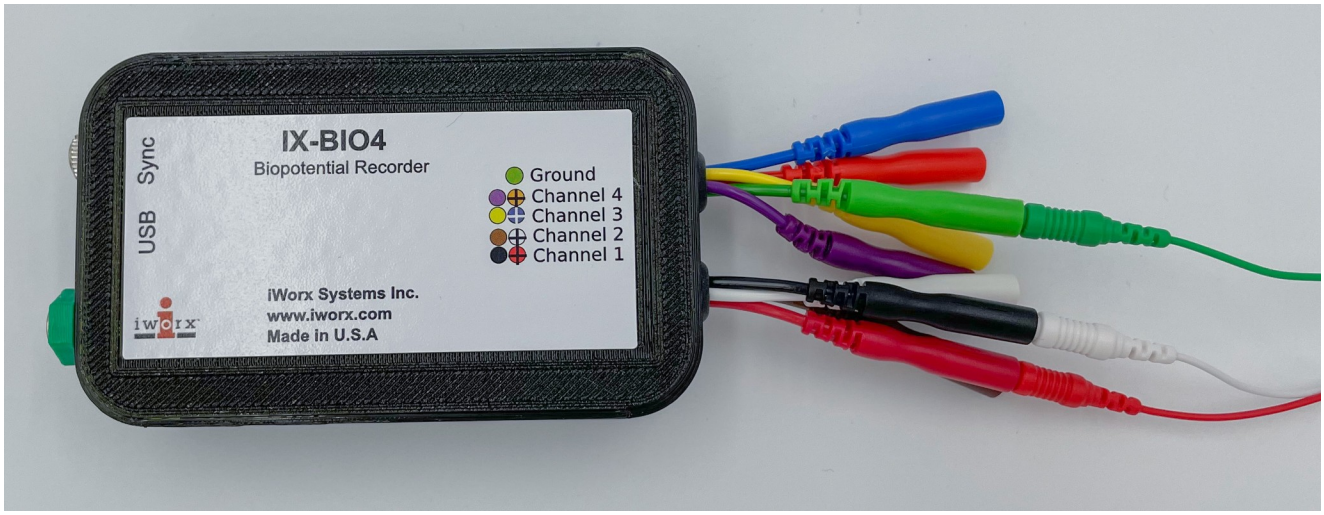


Figure 1: Single channel ECG connections on the IX-BIO4 recorder.
Note: The colors of the electrode wires may not match the colors on the ports of the IX-BIO4.

Equipment Setup for Six Channel

For six channels of ECG, you need to record Lead I and Lead II. The negative connections of both Lead I and Lead II is on the right arm. Instead of using two needle electrodes, you can use the Piggy Back Cable (C-PB-P1) to connect the channel 1 negative and channel 2 negative together. Now you just need one needle electrode for the right arm.

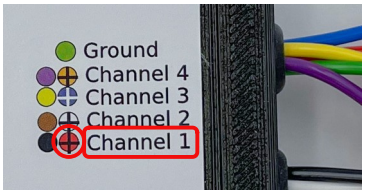
Figure 2: Six channel ECG connections on the IX-BIO4 recorder.
Note: The colors of the electrode wires may not match the colors on the ports of the IX-BIO4.

Preparing the Rodent:

1. The mouse is anesthetized with 2% isoflurane in 100% Oxygen at 300mL/min (following induction in a chamber containing 3-5% isoflurane). The temperature may be monitored and maintained within 37-38 deg C using the SA-TCS-6 or SA-TCS-8 Heater Controller System.

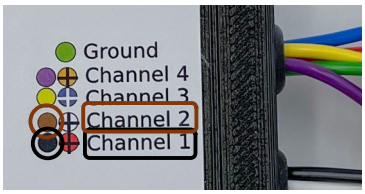
2. Insert the needle electrodes subcutaneously into the limbs of the mouse or rat as follows. Make sure that the needle only penetrates the skin of the animal and is not into the muscle.

The needle electrode plugged into Channel 1 Positive is inserted into the Left Arm of the mouse/rat (LA)

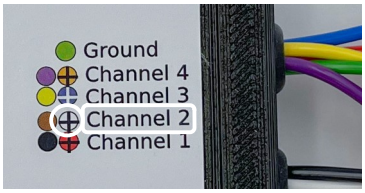


The needle electrode plugged into Channel 1 Negative & Ch 2 Negative is inserted into the Right Arm of the mouse/rat (RA)

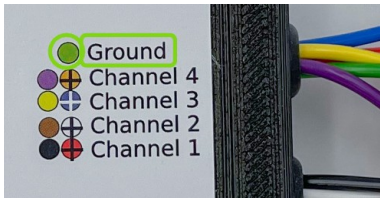
(Piggy Back Cable)



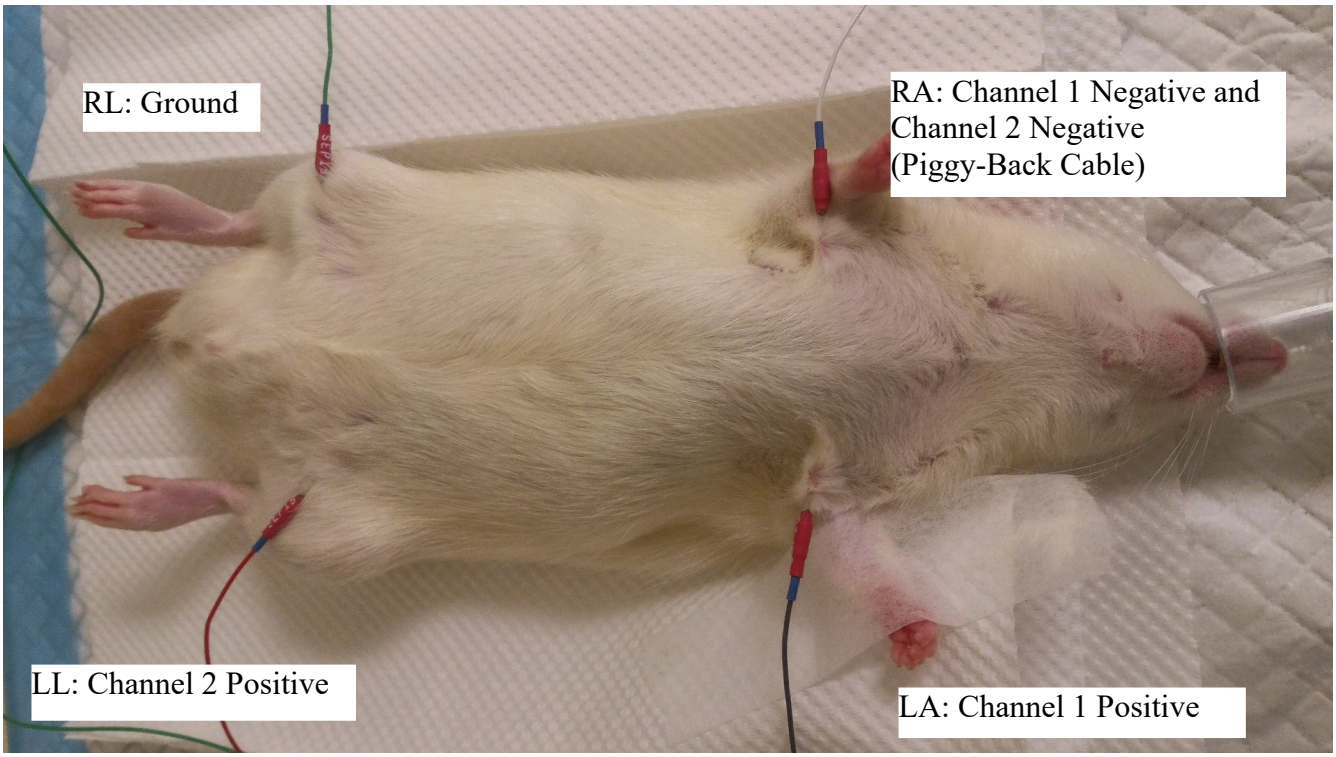
The needle electrode plugged into Channel 2 Positive is inserted into the Left Leg of the mouse/rat (LL)



The needle electrode plugged into Ground is inserted into the Right Leg of the mouse/rat (RL)



***Note**: Good connections to an adequately anesthetized mouse will provide good ECG signal recordings. Insufficient anesthesia may cause the EMG from the animal to be picked up.*



Recording with LabScribe

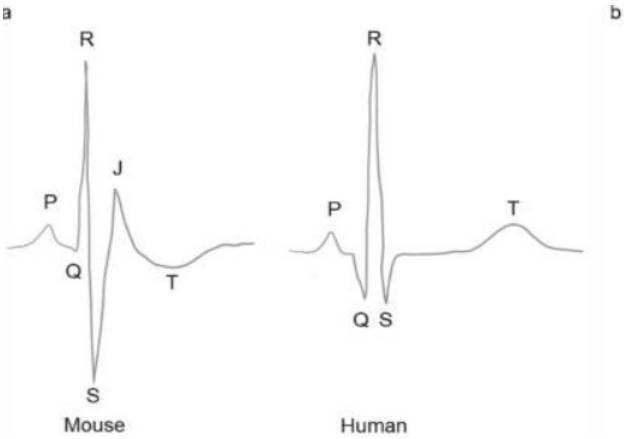
1. Initiate recording by pressing the red icon labeled "**REC**". The icon will now change to black and be labeled "**STOP**".

2. It may take up to 30 seconds for the ECG signal to level out.
3. Select the 'AutoScale all Channels' button at the top of the toolbar.

- This button can be pressed as many times as necessary throughout recording, or the recording can be continually AutoScaled in real time by selecting: View → Display → and Online AutoOffset. This will use the mean values of your data to adjust the trace view so you don't need to keep clicking Autoscale.

4. When the recording is finished Click the "**STOP**" icon.

Typical Mouse vs Human ECG



Segments (ms)	Mouse	Human
PR	30-56	140-220
QRS	9-30	70-100
QT	48-88	400
RR	110-177	857

a. Mouse ECG compared to human ECG.

b. Mouse and human ECG intervals in milliseconds.

Labscribe Software Analysis

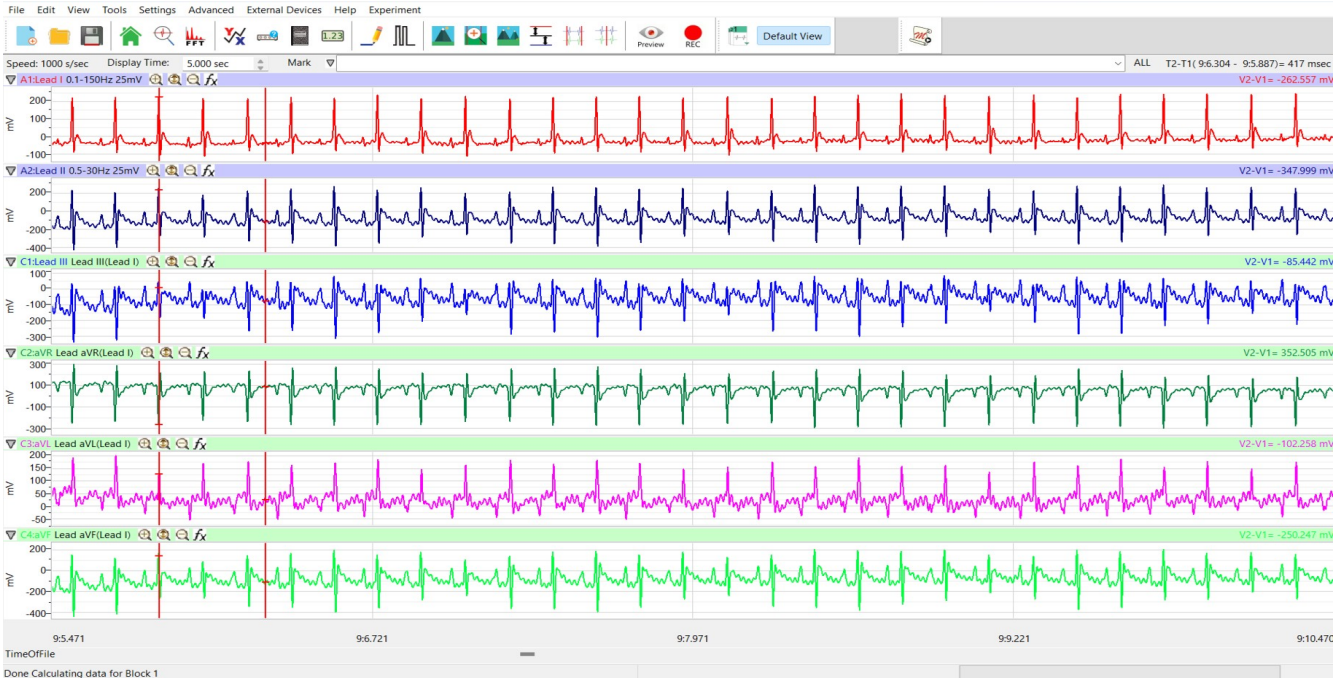
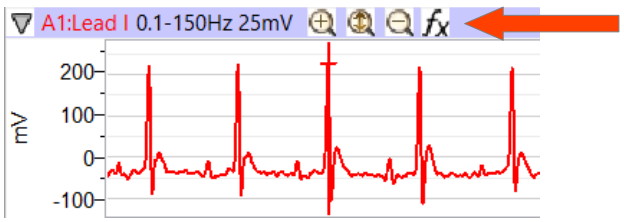


Figure: Measured Leads are Lead I (Red) and Lead II (Navy Blue). Calculated Leads are Lead III (Blue), Lead aVR (Dark Green), aVL (Purple), and Lead aVF (Bright Green).



The fx icon allows for the creation of a new channel with the selected function.

1. Select ‘fx’
2. Select ‘…’
3. Select the type of function you would like to perform on the selected channel.

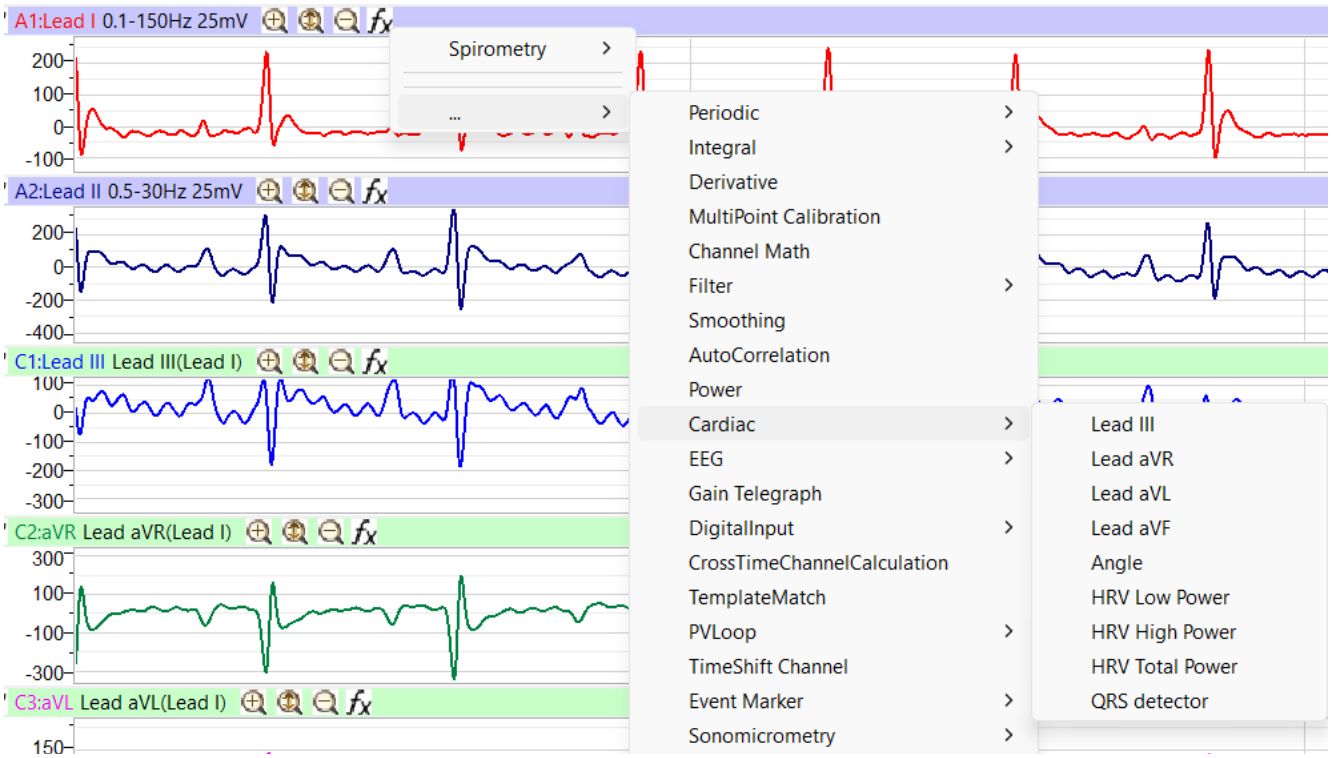
Open the Advanced ECG Analysis Module. Select: Advanced → ECG Analysis → Offline Calculations

Detailed information on ECG Analysis and the Advanced ECG Analysis Module can be found in the LabScribe manual by navigating to:

- In the LabScribe toolbar Select: Help → Help (Manual)
- Section 8.3 ECG Analysis

Basic Cardiac Functions

The Cardiac functions can be accessed through the add function (fx) on the channel of an ECG recording.
Four of the Cardiac functions calculate leads: III, aVR, aVL and aVF from the recordings of Lead I and Lead II. You can specify which channel corresponds to Lead I and Lead II in the Cardiac Setup dialog which will open when a new lead is selected.



Reducing Noise

- Make sure that the heater used for maintaining the animal is a shielded low noise heater.
  - The iWorx SA-TCS-6 or SA-TCS-8 system is designed to minimize noise from the heater.
- To minimize noise from the heater bed, wrap the electrode wires in tinfoil, reduce their length, and braid them to limit power supply noise pickup.
- Utilize a grounding cable to connect the recorder to the wall outlet for better noise reduction.
- Make sure the recording computer is plugged into the wall outlet, not just running on battery power.
- Turn off any excess overhead lights, lamps, and electronics in the lab. Vibrating equipment like pumps, fans, or even nearby footsteps can also affect recordings.