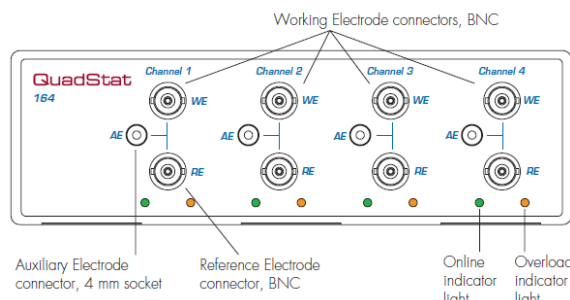


Setting up the eDAQ QuatStat and e-corder for constant potential amperometry (see the eDAQ manuals for further information on hardware and software, also see EDAQ Application Note *EBST 001*)

The Front Panel

The front panel of the QuadStat provides connections for the Working (WE), Auxiliary (AE), and Reference (RE) electrodes. BNC connectors are used for the WE and RE leads. The shields of these connectors are driven to the same potential as the electrode. The connector for the AE lead is a socket for a 4 mm pin.



Electrode Connections

The QuadStat is supplied with appropriate electrode cables, with each lead terminated by an alligator clip to connect to a wide variety of electrodes. The leads are colour-coded to indicate the type of electrode to which they should be attached. The RE and WE leads are shielded to protect the signals from external interference. The shields are driven to the same potential as the electrode to minimize lead capacitance.

Color	Electrode
Yellow	Reference
Green	Working
Red	Auxiliary

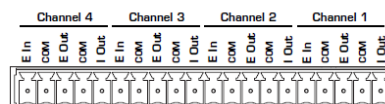
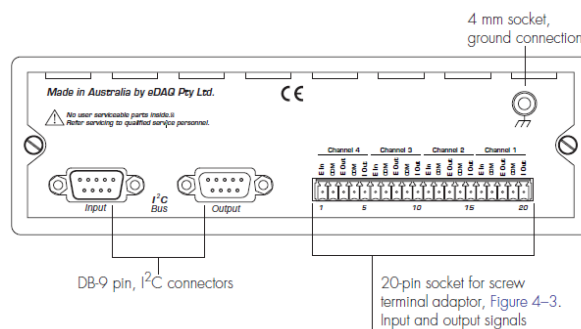
The QuadStat provides two signals per channel: the potential signal (E Out) indicating the potential difference between the working and reference electrodes; and the current signal (I Out) indicating the current flow between the working and auxiliary electrodes.

Overload Indicators

When lit, these indicate that the QuadStat has overloaded, which usually occurs because it has gone out of compliance due to an open circuit (such as an unconnected or faulty electrode), or the resistance is too high in the electrochemical cell. High resistances can be often be encountered when electrodes are fouled by the products of electrolysis reactions.

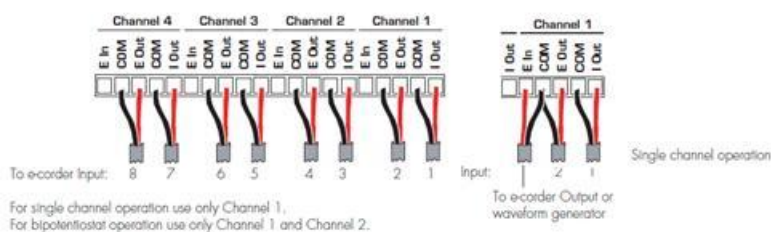
The Back Panel

The QuadStat is supplied with a 20 pin screw terminal adaptor which plugs into the 20 pin socket on the back panel. The pin positions are labelled I Out, E Out, E In, and COM, for each QuadStat channel (Channels 1 – 4). The COM (common) pins are provided for connection to signal ground (black wires of the supplied coaxial cables). You can use any COM pin for the ground connection of any I Out, E Out, or E In signal.



QuadStat rear panel	e-corder front panel
I Out (Ch 1, Ch 2, Ch 3, Ch 4)	Input 1, 3, 5, 7
E Out (Ch 1, Ch 2, Ch 3, Ch 4)*	Input 2, 4, 6, 8
E In †	Output –‡

* It is not always necessary to monitor E Out depending on your experimental requirements.
† Connections to E In are only required if using an external waveform to control the applied potential.
‡ Use Output + to send a signal of reverse polarity to the QuadStat.

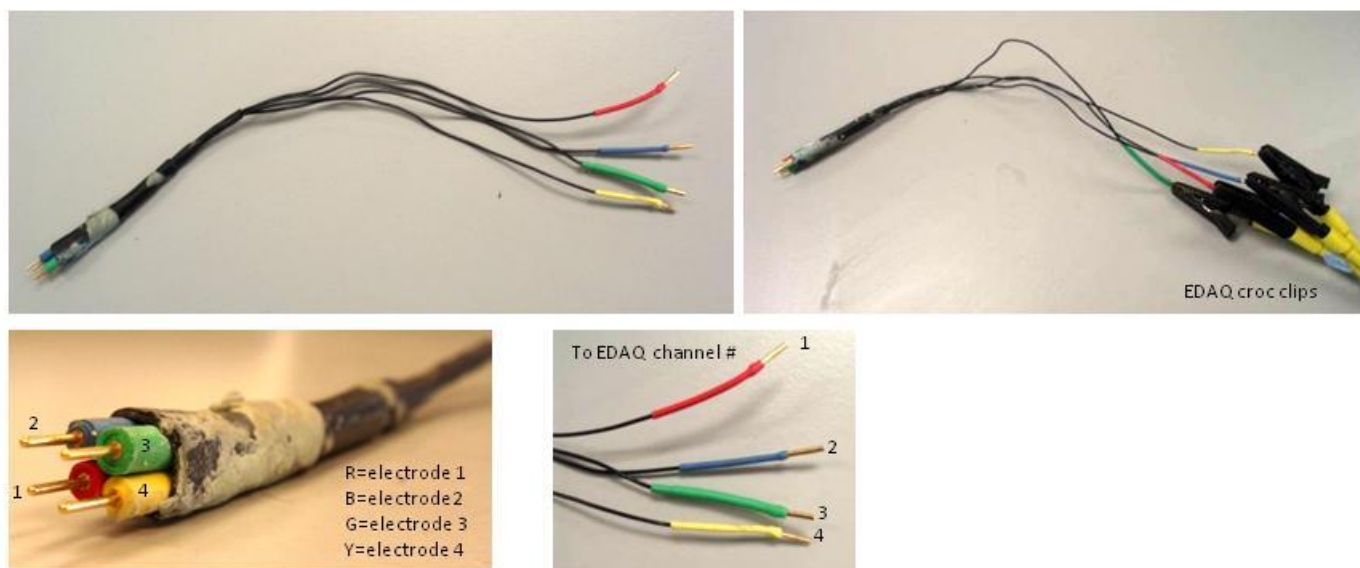


Using a common reference and auxiliary electrode

Often the QuadStat will be used with a single reference electrode, RE, and a single auxiliary electrode, AE (on QuadStat channel 1) with multiple working electrodes (WE) on some, or all, of the other QuadStat channels. Then, to monitor the applied potential signal for Channels 2–4 on each channel with a working electrode, connect the unused AE and RE sockets together.

Connecting the Blue Box Sensors to the QuadStat for in vitro calibrations

The device below was created to connect the gold socket clips to the QuadStat crocodile clips. This connector device is composed of male socket clips soldered to each end of an insulated length of wire, which was colour coded and made more secure with heat shrink. One end of this connector device attaches to the crocodile clips for each corresponding channels on the QuadStat and the other end is inserted into the female socket clips (See Protocol 2 for more detailed description of setting up a cell for in vitro calibrations).



For a simplified connection the gold socket contact can simply be connected to an alligator: BNC cable

In vitro



Sensor



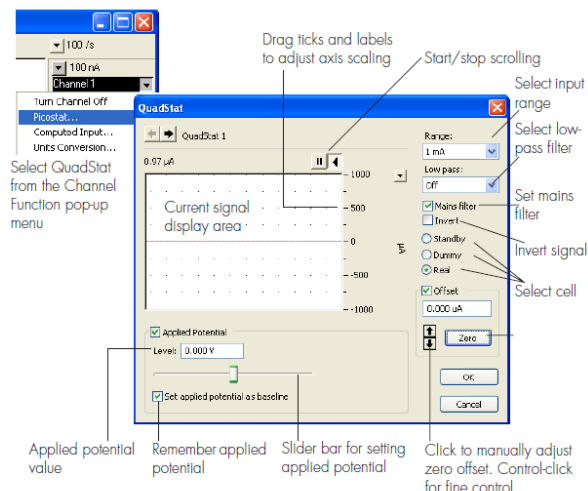
Alligator: BNC cable

Chart Software

Chart is suitable for recording analog voltage signals up to ± 10 V. Install the software and connect the e-corder/QuadStat to a computer. When the e-corder is turned on, and Chart software started, the QuadStat Online indicators (green) should light for every channel connected.

From the Chart software Channel 1 pop-up menu, select the 'QuadStat' command, which accesses the QuadStat control window.

The QuadStat control window allows you to preview the current signal without actually recording the signal to the computer hard disk. (If the menu says 'Input Amplifier' instead of 'QuadStat' then

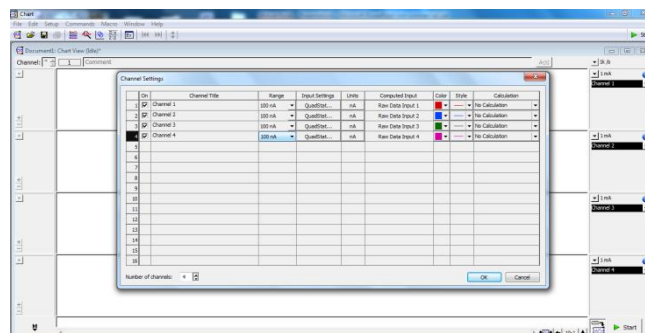


the software has not recognised the QuadStat. Exit the software, check all your connections and try again).

By default, the control window opens with the QuadStat in Standby mode, i.e. with the reference and working electrodes isolated so that no current will flow through your electrodes. To connect to the QuadStat electrode lead wires you must select Real mode. When you click Cancel or OK the QuadStat will revert to Standby mode until recording is started. The Dummy cell mode connects an internal 1 MΩ resistor between the electrodes.

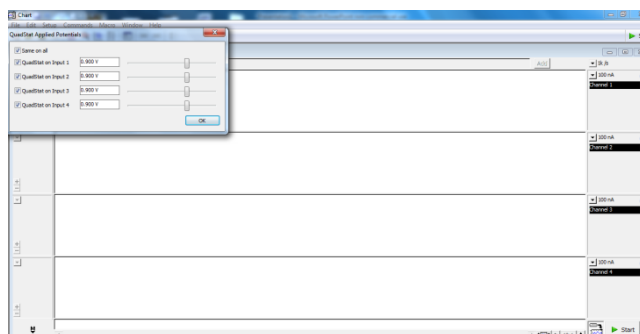
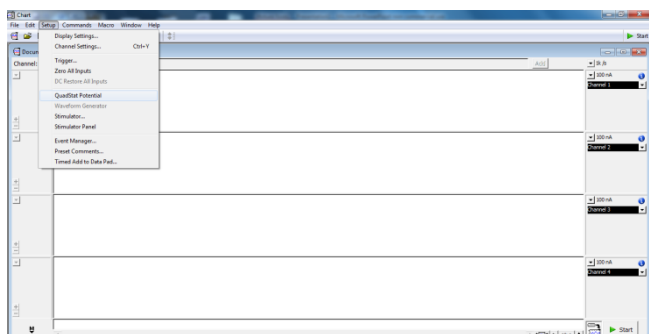
Setting Up the Chart Software

Individual channels in Chart Software correspond to each sensor. In the Setup Menu, go to Channel settings and change the number of channels to 4. Monitoring of potentials throughout each experiment can also be recorded for each individual channel (see the EDAQ manual for more details on connections). Each channel can be given a name by typing in the corresponding box, and ranges can be altered here.



Applying the potential

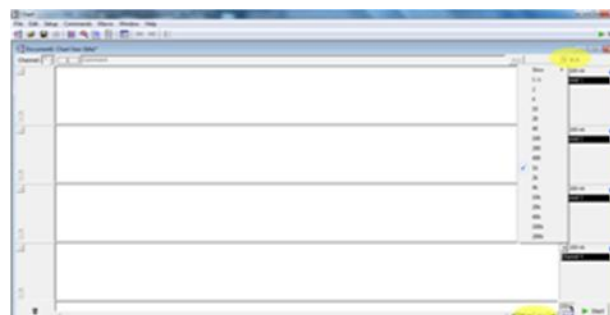
Sensors work by Constant Potential Amperometry, thus a constant potential must be set for each sensor (Oxygen - 650mV, Glucose +700mV, Nitric Oxide +900mV). In the Setup menu, choose 'Quadstat Potential' (screenshot left) which comes up with a control box allowing application of the required potential for each channel. There is an option to apply the same potential to each channel (screenshot right).



In Real mode, the applied potential is set depending on which sensor is being calibrated. The range is also determined by each sensor types baseline characteristics (note, this range may change when calibrations occur)

Sensor	Potential	Range
Oxygen Sensor (see protocol 01)	-650mV (-0.650V)	200nA
OPD Glucose Sensor (see protocol 06)	+700mV (0.700V)	100nA
Nitric Oxide Sensor (see protocol 08)	+900mV (0.900V)	2nA

Set the low pass filter to 50Hz and tick the box for turning on the mains filter, which removes repetitive signals occurring at 50 or 60 Hz which are typical of mains interference. Set the sample size to 4/s and press Start once all the electrodes are connected.



Signals are displayed as a continuous record. You can scroll backwards or forward to review data, compress or expand the time and amplitude axes, zoom in for close inspection, overlay channels, or even plot the data on one channel versus the data on a second channel (XY plotting).

Data Pad

The Data Pad is an internal mini-spreadsheet that helps analyze data by extracting useful parameters (mean, standard deviation, slope, integral, etc.) from selections of data. Select the area you want to analyze and press CTRL+D. This will add that data point to the data pad. This data can then be exported to GraphPad Prism for analysis.

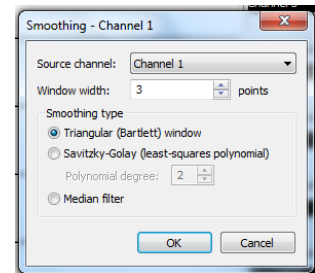
Analysis types for each sensor in GraphPad Prism

Oxygen Sensor	Linear regression for Slope (nA/μM) and linearity (r ²)
Nitric Oxide Sensor	Linear regression for Slope (nA/μM) and linearity (r ²)
Glucose Sensor	Michaelis-Menten analysis for V _{max} , K _m and r ² V _{max} : maximum enzyme velocity K _m : the Michaelis-Menten Constant Linear region slope: V _{max} / K _m (Slope, nA/mM)

It is important to examine your data to decide what kind of analysis you would like to perform: e.g. percent change from baseline, minimum and maximum changes etc. Typical analyses involve t-tests and ANOVAs comparing baseline data with post-treatments. While there are some data analysis tools in the Chart software in the Data pad window (see below), it is often preferred to use a dedicated statistical package such as GraphPad Prism etc.

Smoothing Data

The Smoothing channel calculation allows you to remove unwanted noise or high-frequency components from a waveform. Smoothing works in real-time during sampling, and on pre-recorded data. Two smoothing options are available in each channel dropdown menu: Triangular (Bartlett) Window and Savitzky-Golay (least squares polynomial). The Triangular (Bartlett) Window is a moving average algorithm which smoothes by averaging an odd number of consecutive 2n+1 points of the raw data. Thus, to use this option insert an odd number into the window width box. This number can be increased or decreased until you are satisfied with the level of smoothing.



Connecting the Blue Box Sensors to the QuadStat for in vivo recordings ('tethered')

In vivo: Once implanted by stereotaxic surgery, sensors are inserted into the underside of a six channel pedestal which is cemented in place. Following surgical recovery, the six pin cable is inserted into the topside of the six channel pedestal and is connected to the EDAQ Quadstat via BNC cables. Four working electrodes can be implanted simultaneously in conjunction with a reference and auxiliary electrode. Surgical protocols are available on request from Blue Box Sensors. A commutator (swivel) is required for this setup. We recommend the Bioanalytical Systems, Inc Rat Urns. Contact us if you require a quote or further information.



Once the sensors are connected to the Quadstat, recording can begin by pressing the Start button in the Chart software window. Recording can be monitored 24/7 and data analysis capabilities are inbuilt in the Chart Software.

In vivo recording Setup

