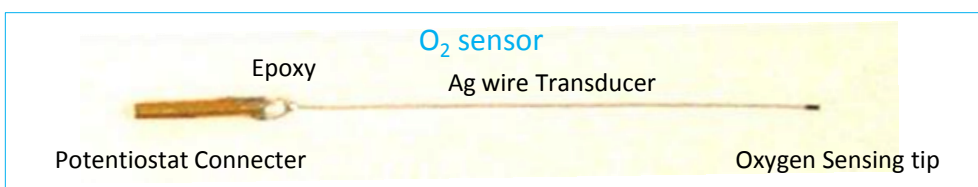


Blue Box Oxygen Sensors User Manual

About the Sensor

The carbon paste oxygen (O_2) and polymer O_2 sensors have been developed to allow implantation in the brain, facilitating long-term *in vivo* and real time measurements of O_2 in freely moving animals. The sensors are less prone to surface poisoning and are stable over months for *in vivo* (tethered or telemetry (carbon paste)) applications. There is good evidence that oxygen levels in brain ECF are related to cerebral blood flow. Oxygen Sensors are based on the electrocatalytic reduction of oxygen at the electrode surface. Measurements are made using constant potential amperometry by applying a constant voltage to the sensor (-650 mV), where the resultant measured current is proportional to the dissolved O_2 concentration. The dimensions of CPEs are 200 μ m internal diameter and are typically supplied as 4cm in length. If you require a different length for your specific purposes, please contact us. Constant potential amperometry requires a potentiostat and data acquisition system. We recommend the use of eDAQs popular range of potentiostats, e-corders and Chart software. Contact us for more details.



Handling the sensor

The sensor should not be autoclaved and should be handled by the gold socket. Care should be taken when handling the sensor so as not to disrupt the carbon paste loading and not to damage the wire. Care should also be taken around the epoxy junction. The transducer wire can be bent gently to suit your needs.

Sensitivity

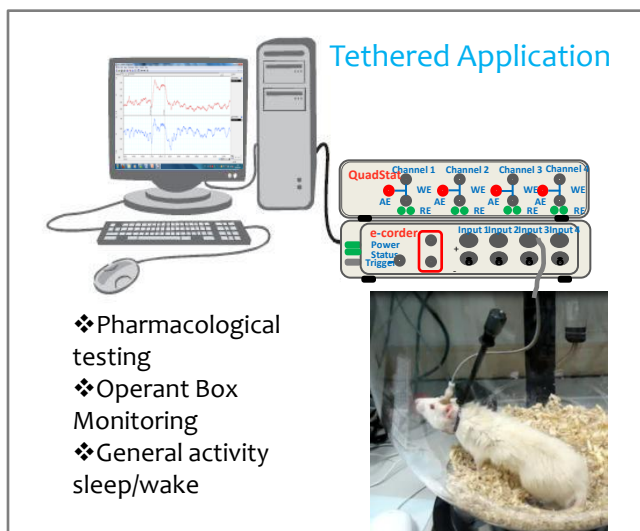
Each sensor is calibrated individually and the exact calibration constant is printed on the box the sensor is shipped in (typically \sim 1.5nA/ μ M for dissolved O_2).

The sensitivity remains stable for long periods (up to 6 months) of implantation. There may be a reduction in this sensitivity depending on length of implantation and surgical variations. As a result, post-implantation calibrations are recommended for accurate absolute O_2 levels (see below). If post-calibration is not feasible in your lab, the sensors can be shipped back to Blue Box Sensors for post-calibration.

The response to changes in oxygen concentration is immediate (response time < 1 sec) and linear between 0 to 1200 μ M.

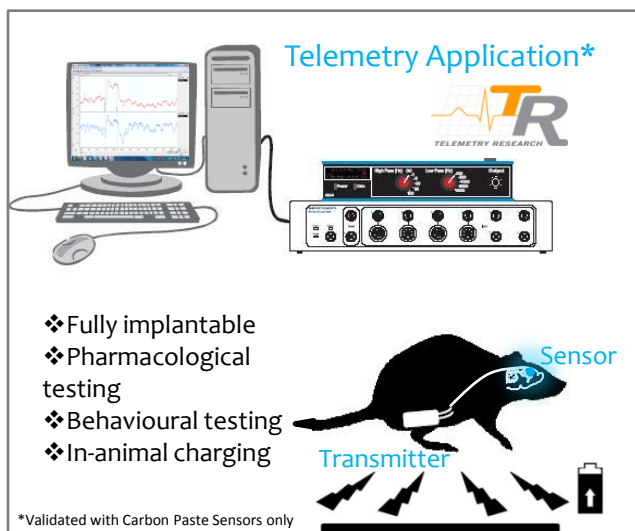
Blue Box Carbon Paste O_2 sensor	
Amperometry Potential	-650mV
Sensitivity (average)	-1.5nA/ μ M
Diameter	200 μ m
Length	Custom made
Response time	< 1 sec
Stability <i>in vivo</i>	3-6 months
Storage	4°C

Tethered Application



- ❖ Pharmacological testing
- ❖ Operant Box Monitoring
- ❖ General activity sleep/wake

Telemetry Application*



- ❖ Fully implantable
- ❖ Pharmacological testing
- ❖ Behavioural testing
- ❖ In-animal charging

*Validated with Carbon Paste Sensors only

Blue Box Carbon Paste/ or Polymer Oxygen Sensor

Using the Oxygen Sensor - *In Vivo*

The sensor design is based on a typical 3 electrode electrochemical detection system. The O₂ sensor is the ‘working electrode’. The ‘reference electrode’ is a silver wire which acts as a reference to which the potential is being set, and an ‘auxiliary electrode’ that completes the electrical circuit with the working electrode. The O₂ sensor potential is set at -650 mV relative to the silver reference electrode.

Auxiliary Electrode

Reference Electrode



The microscrew on the end is inserted into the skull. This screw forms one of the four support screws designed to stabilize the head-stage pedestal.

The exposed silver wire at the tip is inserted into the cortex below the dura. The coiled (bent) portion is designed to sit on the skull.

Implantation

Upon request we can provide surgery guidelines for implantation of the electrodes in the brain for either tethered or wireless (telemetry: carbon paste sensor) applications. When each electrode is inserted into the correct location (O₂ sensor in desired brain area, reference electrode in the cortex and auxiliary electrode in the skull) apply dental cement to stabilize their positions.

Apply a potential of -650mV and the sensors should be given approximately 30 mins to stabilize after the voltage has been applied to ensure a stable baseline.

Connection of Sensors to a potentiostat

In vitro: the gold socket contact can simply be connected to an alligator clip.

In vivo (freely moving): Once implanted, 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 an EDAQ potentiostat (or similar) via BNC cables. Four working electrodes can be implanted simultaneously in conjunction with a reference and auxiliary electrode for the tethered application. Surgical protocols are available on request from Blue Box Sensors.

In vitro (calibrations)



Sensor



Alligator: BNC cable (or potentiostat specific connection)

In vivo (freely moving tethered application)



Sensor



Pedestal



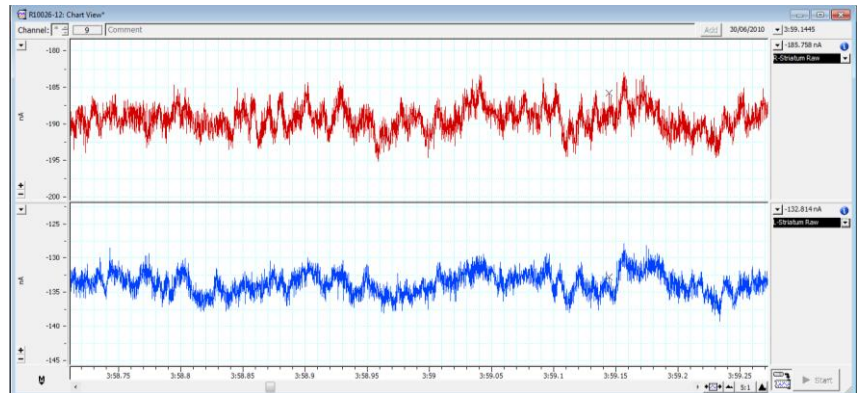
Six pin cable

Blue Box Carbon Paste/ or Polymer Oxygen Sensor

Validating the Oxygen sensor - *In Vivo*

After surgery, when the animal is recovered from anaesthetic, place in the home cage. When the regains mobility, connect the head stage pedestal to the potentiostat. Typically, it takes 2-4hours for the signal to fully stabilise and recordings can begin. Save a new file for each day to prevent the file size from becoming too large.

Typical example of raw data recorded from two oxygen sensors implanted in the striatum area of the brain. The potential is -650mV. An oxygen signal fluctuates with general activity. A typical baseline response is ~200nA but varies with different surgical success. The raw data below shows the typical baseline from an oxygen sensor when it has settled.

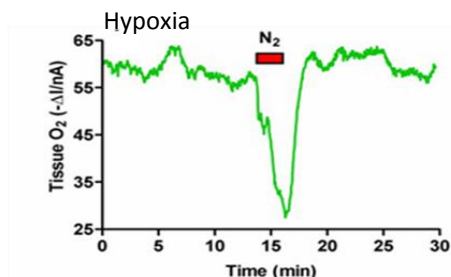
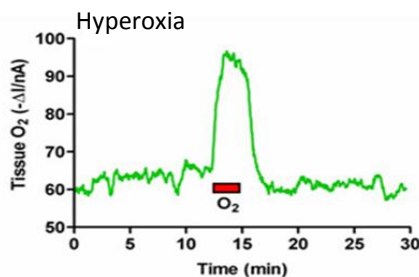


Hyperoxia (O₂) and Hypoxia (N₂)

Inducing hypoxia and hyperoxia are suitable for verifying the response of CPEs to changes in brain tissue O₂ *in vivo*.

Hyperoxia: Apply a gentle flow rate 100% Oxygen in the vicinity of the rat snout for 3 mins. Take care not to touch the whiskers as this will result in activation of the whisker barrel cortex. There is typically a change in current of approximately 27.6 ± 2.8 nA (Bolger & Lowry, 2005), although this varies depending on surgical success and sensor sensitivity. Once the oxygen gas is removed, the signal will rapidly return to baseline levels. Wait for at least 2 hours before applying nitrogen.

Hypoxia: Apply a gentle flow rate of Nitrogen in the vicinity of the rat snout for 3 mins. Take care not to touch the whiskers as this will result in activation of the whisker barrel cortex. The rat may try to avoid the N₂ gas, so it is necessary keep the gas supply near the snout as the animal moves around the home cage. There is typically a change in current of approximately 27.2 ± 4.2 nA (Bolger & Lowry, 2005), although this varies depending on surgical success and sensor sensitivity. Once the nitrogen gas is removed, the signal will rapidly return to baseline levels.



Source: Bolger & Lowry, *Sensors* 2005, 5, 473-487

Lifetime of the sensors

The O₂ sensors show stability over long periods of repeated recording. The sensors have been tested to be stable for three to six months when implanted in the brain. Degradation of the sensor by fouling will show as a gradual decrease in baseline current over time. Acute failure of the sensor is more likely to show up as a large increase in baseline levels. This is because a damaged sensor will start to pick up interferences such as ascorbic acid. The sensors are single use, that is, after removal from the animal they should not be used again. Sensors can be stored at 4°C. The shelf life is half a year from the date of delivery.

Blue Box Carbon Paste/ or Polymer Oxygen Sensor

Calibrating the sensor - *Ex Vivo*

Sensors are already supplied pre-calibrated with the sensitivity value printed on the back of the box in nA/ μM . Should you wish to calibrate the sensors post implantation, known concentrations of oxygen can be achieved with nitrogen gas, air and oxygen gas which will give a 3-point linear calibration plot. A reference electrode such as a Saturated Calomel electrode is required as well as an auxiliary electrode (ideally made of silver or platinum).

Cell Setup:

Place a glass vial with 10ml PBS (pH 7.4) in a retort stand for stabilization during recording and seal with a lid which has hole ports for inserting the electrodes and tubing from the gas cylinders.

Nitrogen ([0 μM] O₂): Nitrogen gas can displace the carbon paste at the active tip of the electrode, the PBS solution must first be deaerated with O₂-free N₂ gas for ~30mins to achieve a 0 μM O₂ concentration resulting in a current response of ~0nA. Perform this before placing the carbon paste electrodes in the PBS.

Remove the N₂ supply from the buffer and maintain the gas supply above the buffer within the cell to record the current during the quiescent period. Place the sensor in the PBS along with a reference and auxiliary electrode, connect the electrodes to the potentiostat and apply the constant potential (-650mV). Ensure the electrodes are in the solution, are not touching each other and are devoid of bubbles. Record the quiescent baseline for 10 mins to allow the electrodes to settle.

Air ([240 μM] O₂): Use an air pump (eg Rena Air 200) to bubble atmospheric air through the cell for an O₂ concentration of 240 μM resulting in a current response in the region of -500 \pm 100nA. Allow 30 mins for the buffer to become saturated. Remove the air from the buffer, but maintain the supply above the buffer and within to cell to record the current during the quiescent period.

Oxygen ([1200 μM] O₂): Finally, achieve an O₂ concentration of 1200 μM by bubbling pure O₂ gas through the buffer solution which causes saturation of the buffer solution within 10mins and results in a current readout of ~-2000 \pm 50nA. Again, remove the O₂ supply from the buffer, but maintain the supply above the buffer and within to cell to record the current during the quiescent period.

Example of raw data for an oxygen calibration

The decrease in current during the quiescent period following gas administration is a result of the removal of the forced convections due to gas bubbling. Only take steady state currents in the absence of convection into account when calculating the regression analysis.

These concentrations are used to allow regression analysis where all calibration plots are linear and the slope (nA/ μM) is used as an index of sensitivity of the CPE oxygen sensors.

An example of the current (I)-concentration ([O₂]) profile for a carbon paste electrode oxygen sensor carried out at -650mV vs SCE in response to buffer (PBS, pH 7.4) saturation with N₂ gas, air and O₂.

