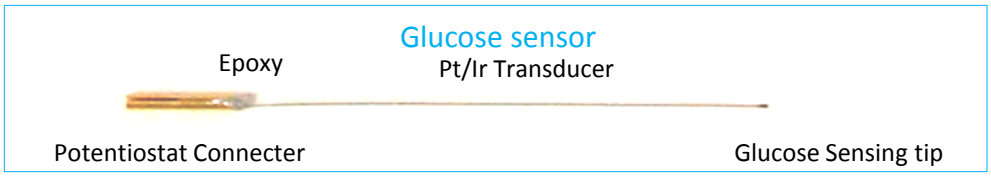
Blue Box Glucose Sensor User Manual

About the Sensor

The Glucose sensors have been developed to allow implantation in the brain, thus facilitating long-term in vivo and real time measurements of glucose. The sensors are based on the immobilisation of glucose oxidase (GOx) on Platinum/Iridium (Pt/Ir) wire with a stabilising polymer layer. These are less prone to surface poisoning, are highly specific and are stable over several weeks for in vivo applications. Measurements are made amperometrically using constant potential amperometry by applying a constant voltage to the sensor (+700 mV). The dimensions of glucose sensors are 175µm external diameter and are typically supplied as 3cm or 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, ecorders 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 sensitive tip 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.

The voltage on the sensor should not be switched to negative values, as this will damage the sensor.

Sensitivity

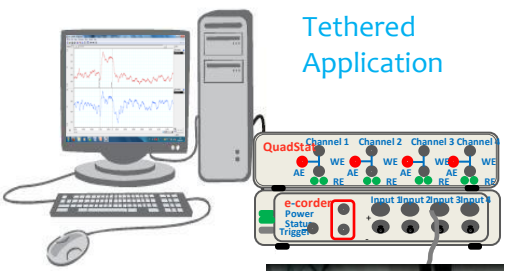
Each sensor is calibrated individually and the exact calibration constant is printed on the box the sensor is shipped in. The response to glucose concentrations (1mM-1000mM) is immediate (response time < 1 sec) and linear.

The sensitivity of the sensor remains stable for several weeks (up to 4 weeks) of implantation. There may be a reduction in this sensitivity depending on length of implantation and surgical variations. As a result of this, post-implantation calibrations are recommended for accurate absolute glucose 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.

Lifetime of the sensors

The Glucose sensors can be stored at 4°C. It is recommended that the sensors are used immediately due to the possibility of enzyme degradation. The sensors have been tested to be stable for 4 weeks when implanted in the brain. Degradation of the sensor by fouling will show itself 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 interferents such as ascorbic acid. The sensors are single use, that is, after removal from the animal they should not be used again.

Blue Box Glucose sensor	
Amperometry Potential	+900mV
Sensitivity (average)	1.5nA/ µM
Detection Limit	3.5µM
Diameter	175µm
Length	Custom made
Response time	< 1 sec
Stability in vivo	1months
Storage	Room temp.



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

Blue Box Glucose Sensor

Using the Glucose Sensor - *In Vivo*

The sensor design is based on a typical 3 electrode electrochemical detection system. The Glucose 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 Glucose sensor potential is set at +700 mV relative to the silver reference electrode.

Auxiliary 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.

Reference Electrode

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 (NO 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 +900mV and the sensors should be given between 2-8 hours 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 (or similar). 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

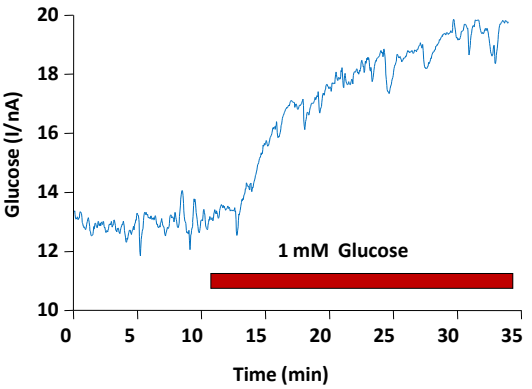
Six pin cable

Blue Box Glucose Sensor

Validating the Glucose sensor - *In Vivo*

The most definite way to validate the Glucose sensor *in vivo* is to perform pharmacological tests either by systemic interperitoneal injections or by co-implanting a microdialysis probe for local infusion in the vicinity of the sensor. Typically to induce glucose response, simply glucose can be used. Physiological stimulation by tail pinch is also known to induce an increase in glucose concentration in the brain.

Typical example of data recorded from a glucose sensor implanted in the striatum area of the brain. The potential is +700mV. A typical baseline response is~20nA but varies with different surgical success. The raw data shows the rapid response of the sensor to local infusion of glucose or to physiological stimulation with a tail pinch



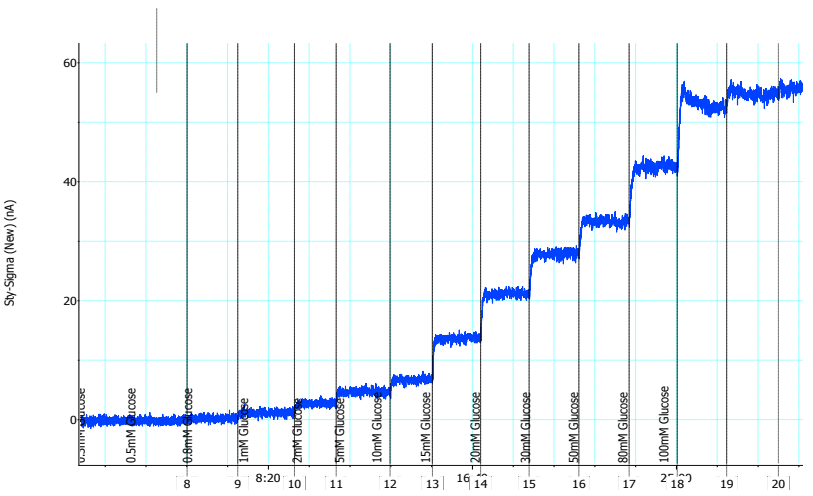
Lowry et al., (1998) J Neurosci Methods. 79(1):65-74

Calibrating the sensor - *Ex Vivo*

Each sensor is individually calibrated with known concentrations of Glucose (0-100mM) and provided with a Sensitivity (Slope, nA/µM) value. This calibration information is printed on the back of the box each sensor is supplied with. The data below shows an example Glucose sensor response to known concentrations of glucose applied to the sensor which is immersed in 10ml PBS in a stepwise manner from 0-100mM concentrations and analysis generated from this sensor response, showing the Slope and linearity.

Calibration Injection Protocol (1M Glucose (9.01g/ 50ml dH_2O)

Glucose Concentration	Injection (µl) added to 10ml PBS	Total µl
0.3mM	3µl	3µl
0.5mM	5µl	8µl
0.8mM	8µl	16µl
1mM	10µl	26µl
2mM	10µl	36µl
5mM	40µl	76µl
10mM	50µl	126µl
15mM	50µl	176µl
20mM	50µl	226µl
30mM	100µl	326µl
50mM	200µl	526µl
80mM	300µl	826µl
100mM	200µl	1026µl



We can not guarantee the lifetime and sensitivity of the sensor for repeated use, as the sensor has not been characterized as such. Technically the sensor can be used a number of times if not exposed to lipids or proteins and washed after use with dH_2O. However, the sensitivity may change due to adsorption of analytes in the electrochemical cell. The surface of the electrode will be slightly altered with each application and re-application of the potential and also degradation of the enzyme. The simplest test for the integrity of the electrode is by measuring its response to ascorbic acid (the sensor is designed to reject ascorbic acid as well as all other interferents). If the response to ascorbic acid is similar to the calibration data provided with the electrode, the sensor is still mechanically intact.

www.blueboxsensors.com
E-mail: info@blueboxsensors.com