



sarissaprobe™-ATP, Instructions for Use

The sensors are fragile –handle with care and do not touch or knock the sensing tip. Take care when removing from packaging –use a pen or forceps to lever the sensor up out of the packaging.

Storage

Store in Fridge at 2-8 C before use.
Operation of sensor after use-by date is not guaranteed.

Rehydration procedure

Prepare Buffer A: containing 10 mM NaPi buffer, pH 7.4, 100 mM NaCl, 1 mM MgCl₂ and 2 mM glycerol.

Pour a suitable volume of Buffer A into rehydration pot, and immerse the tip of the sensor into the buffer. Leave for at least 10 min.

Following rehydration do not attempt to re-dry the sensor.

Keep exposure of sensor in the air to a minimum –preferably no more than 30s at a time as prolonged exposure to air can result in loss of sensitivity. If prolonged exposure is anticipated, e.g. due to slow loading of sensor into apparatus briefly dip sensor into a 5 M solution of glycerol to prevent drying of enzyme layer.

Once a sensor has been rehydrated, store in Buffer A at 2-8 C. Wet storage of sensor following rehydration is guaranteed for 5 days. Care should be taken to avoid bacterial contamination of the sensors –we recommend sterile filtering or autoclaving Buffer A.

First calibration and sensor test –in vitro

Fill calibration pot with 30 ml of Buffer A. Insert sensor and Ag/AgCl reference electrode into sockets provided in the lid of the pot and then dip into the pot.

Connect Ag/AgCl reference electrode and sensor to 2-electrode potentiostat.

Turn on potentiostat and polarize to +500 mV.

Allow sensor current to asymptote –should take around 5 min. Take zero reading.

Make up a fresh solution of 40 μM ATP in Buffer A. Fill a syringe with 10 ml of ATP solution and inject into the pot through the central hole in the lid. The addition of the 10 ml of ATP solution will give a final concentration of 10 μM. Allow sensor current to give a typical response. Response amplitudes vary with size of sensors (see table 1).

Cycling –this will improve sensor sensitivity by up to 3 fold. Cycle the sensor from –500 mV to + 500 mV and back at a rate of 100 mV/s for 10 cycles. Then polarize to +500 mV for calibration.

Remember to place sensor back in storage pot or onto the recording setup quickly –do not allow the sensor to dry.

Note -if the user has access to a flow system, all calibration and testing of the sensor can be performed in this system.

Calibration -in situ

All physiological salines used for sensor experiments should contain 2 mM glycerol –this is a cosubstrate required for sensor operation.

Regular calibration during an experiment is necessary to monitor for possible loss of sensor sensitivity after contact with tissue. Loss of sensor activity can be partially restored by cycling as described above.

Sensors should be calibrated out of contact with tissue –as the tissue impedes free access of exogenous ATP to the sensor surface.

If the tissue is in a flow chamber, the simplest procedure is to withdraw the sensor from the tissue and allow a test solution of ATP (e.g. 10 μM to flow through).

Note that calibration of the sensor in a physiological buffer may differ from that recorded in Buffer A due to the different ionic composition of the buffers. The sensor should always be calibrated in a saline of the composition use in the experiment with tissue.

Experimental use

Setting up

A high quality micromanipulator mounted on a magnetic base positioned so that the sensor can access the tissue and structures of interest is essential. This should be arranged appropriately *before* mounting the sensor as prolonged exposure of sensor to air can kill its activity. If a **sarissaprobe™ -ATP-Null** sensor is also to be used simultaneously, this too should be mounted on a similar manipulator and positioning optimised prior to mounting the sensors.

An Ag/AgCl reference electrode should be present in the bath (or preparation) and connected to the potentiostat. This reference electrode can be the same as the bath ground or reference used for other electrophysiological equipment such as extracellular amplifiers, patch clamp amplifiers etc. We recommend that the Ag/AgCl reference should have an agar bridge if used with a flow system, but this is not essential. If an ATP sensor and reference sensor are used simultaneously, connect the reference points on the potentiostat together and to a single Ag/AgCl electrode in the bath.

Remember to include 2 mM glycerol in the physiological buffer.

Polarization of sensors

Once everything is ready, insert sensors into holders and as quickly as possible move them into the bath or recording chamber so that they are immersed in physiological saline. Switch on potentiostats (polarization potential 500 mV) and check that the sensors are polarizing –it is best to have the potentiostat within range (not overloaded) to ensure correct polarization of the sensors. It can take several minutes sometimes 15-30 minutes for polarization to occur fully. This usually speeds up with further use of the sensor. When you are happy with the baseline current you are ready to proceed with your measurements. A calibration in the recording chamber is probably a good idea.

Criteria for re-use of sensors

Use of the biosensors with tissue inevitably leads to gradual loss of sensitivity from the sensor. This may be for a variety of reasons –physical damage, fouling of the sensor surface by proteins, degradation of the enzymes within the sensor by proteases released from the tissue. The rate and extent of loss depends on how the sensors are used and with what tissue.

Sensitivity can be retained to the greatest extent if you treat the sensors carefully and wash them in buffer to try and

remove any adsorbed proteins. Remember to store them in the fridge overnight when not in use. Also remember to limit their exposure to air as much as possible.

Protein fouling of the sensors can be reduced by briefly dipping them in 50% PEG20k (in pure water or buffer A).

Cycling the sensors from -500 mV to plus 500 mV and back, for 10 complete cycles at a rate of 100 mV/s will regenerate some of the lost activity.

Ultimately the user has to decide whether the sensors retain sufficient sensitivity for further use. As a rough guideline we would suggest that if the response to 10 μM ATP dips below 1 nA for a 2mm / 50 μm sensor, or 0.25 nA for a 0.5 mm / 50 μm sensor (see table 1), the sensor may be insufficiently sensitive to use, unless the investigator is confident that he/she is attempting to measure an inherently large signal.

Tips for effective use of sensors

Unstable signal

Slow cyclical instability of signal may well be caused by changes in the level of the recording chamber. The cure for this is to carefully balance the inflow and outflow to the chamber to ensure the level stays constant.

The sensors are sensitive to noise generated within a flow system. It is important to keep tubing as clean as possible and to isolate the inflow and outflow tubes from the recording bath by means of drip chambers. If the sensor record is marked by sudden apparently random glitches these may well be created in the flow system and replacement of old tubing/drip chambers by new items may well cure the problem.

In flow systems that involve heated chambers, there may be out-gassing within the recording chamber and bubbles may stick to the sensors and recording electrodes. This can also be a source of glitches on the sensor signal. Preheating of the saline prior to it entering the chamber helps to minimize this. Gentle dislodging of any offending bubbles also helps.

Very occasionally, the sealing around the Pt wire of the sensor may lose its integrity with prolonged use. This will give rise to sudden fluctuations of the sensor current. To eliminate this as a possibility as a cause polarize the sensor in a still chamber (to eliminate any flow artefacts) to see whether the noise is still present or disappears. If it is still present under these conditions, the sensor has come to the end of its useful life.

Noise

Lowest noise will be obtained if the sensors are used within a Faraday cage and on a metal baseplate. The methods for noise reduction for sensor recordings are very similar to those used with any electrophysiological setup.

Controls

The sensor is selective for ATP over ADP, UTP and adenosine. However there are known electroactive interferences such as ascorbate, 5HT/dopamine/noradrenalin and urate. The sensors have an inner permselectivity layer that screens the majority of signals from these electroactive interferences. Nevertheless, the onus is on the experimenter in every application of this biosensor technology to demonstrate that the signal originates from the release of ATP rather than a non-specific interferent.

One important control is to omit glycerol. As the enzymatic layer can retain glycerol, this control should be performed after extensive washing in glycerol-free saline and with the demonstration that in the absence of added glycerol the sensor no longer responds to ATP. Recordings from tissue made in the absence of glycerol should show no signal, any remaining signals would be non-specific and due to interferent. When glycerol is washed back, the signals due to ATP release

will be present and can thus be discriminated from the interferences.

Using the sarisaprobe™-ATP-NULL sensor as a control

The ATP-Null sensors are made from the same materials as the ATP sensors, but lack the detecting enzymes. They thus have the same outer layer and inner permselective screen as the ATP sensors but lacking the enzymes cannot detect ATP. The Null sensors can thus be used as a further control –no specific signals should be present on the Null sensors. Ideally simultaneous recordings from the reference and ATP sensors will give greater confidence

Differential measurements

If the ATP and Null sensors can be placed identically in the tissue with respect to the source of ATP release, a differential measurement can be performed. That is the signal on the Null sensor can be subtracted from the signal on the ATP sensor to give a "pure" ATP signal. This can be done online with a simple DC differential amplifier. If the data is acquired to a computer through an A/D interface, the subtraction can be done offline digitally.

If the experimenter uses the Sycopel Duostat, this instrument provides an online difference signal during the experiment.

Insertion and placement of sensors

Care needs to be taken with the insertion and placement of the sensors. This is especially true if the experimenter desires to make valid differential recordings. We recommend the use of high quality manipulators that will give smooth vibration free movement of the sensors. Insertion of the sensor into the tissue should be performed at a slow to moderate speed to minimize damage to the tissue. Preferably the sensor should be inserted axially to minimize tearing of the tissue.

Interpretation of sensor calibration

The conversion of sensor current to analyte concentration by means of calibrating the sensor against known concentrations of ATP is not necessarily as straightforward as it might seem. The sensor detects ATP over its entire surface area. Consider the case where the analyte is released in a punctuate manner and diffuses to only a small proportion of this sensor surface area. Conversion of the current measured to analyte concentration, on the basis of a calibration measurement that is obtained by exposing the entire sensor to ATP, will not be valid. An example will help to make this clear: if calibration of the sensor gives 5 nA per 10 μM ATP and during an experiment a signal of 2.5 nA is measured, this could represent 5 μM ATP if measured over the whole sensor surface area or for example 50 μM ATP if measured over a tenth of the sensor surface area.

If the experimenter can obtain *independent* evidence as to the area of ATP release, it is possible to scale the calibration according to exposed surface area. In any case caution should be used when making quantitative interpretations of sensor measurements.

Table 1

	Standard Minimum	To expire
0.5mm, 50 μm	1nA	0.25nA
2mm, 50 μm	4nA	1nA
0.5mm, 25 μm	0.5nA	0.1nA