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Ion Selective Electrode for TPP^+ and O2k high-resolution respirometry

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Tetraphenylphosphonium (TPP^+) accumulates in the mitochondrial matrix as a function of the mitochondrial membrane potential. The TPP^+ electrode is an ion selective electrode (ISE). The signal [V] is linearly dependent on the logarithm of free [TPP^+].

The ISE Module (Oroboros Instruments) consists of separate measuring and reference electrodes. The ISE are inserted through the stoppers into two chambers of the Oxygraph-2k and connected to the potentiometric channels of the O2k-MultiSensor system, for simultaneous recording of oxygen and TPP^+. The ion selective membrane is mechanically mounted and can be exchanged comparable to the membrane of polarographic oxygen sensors. The membrane composition is optimized to increase signal stability and minimize side effects by chemical substances such as ethanol and ADP. Correction for residual side effects is integral to high-resolution measurement of TPP^+. A high-quality inner glass electrode is inserted into the electrode housing of the ISE, which is made of PVDF to minimize oxygen diffusion and carry-over of inhibitors and uncouplers dissolved in ethanol or DMSO. The electrode is integrated into the O2k chamber with minimal oxygen diffusion.

TPP^+ electrode	Raw signal @ 0 TPP^+ [mV]	Sensitivity @ 1-3 µM TPP^+ [mV/dec]	Critical [TPP+] @ 40 mV/dec [µM]	|Drift| of raw signal [mV/s]
Mean ± SD	-196 ± 14	53 ± 5	0.7 ± 0.3	0.0007
N	96	99	82	99

Table 1: Typical performance parameters of Oroboros TPP^+ electrodes. The critical sensitivity is defined at 40 mV/decade [mV/dec]. At this sensitivity, the critical [TPP^+] was 0.7 µM. The absolute value of drift was measured in all calibration runs in the range 2-5 µM TPP^+.

A long-term study over 3.5 months was designed to evaluate the long-term stability of 10 TPP^+ electrodes without change of membranes. A standard rinsing procedure for TPP^+ electrodes was developed for routine experiments, consisting of a sequence of water, 99% ethanol, and water, which is necessary when using inhibitors and uncouplers.

Calibration tests were performed in Oxygraph-2k chambers with MiR06 at 37 ºC, at a stirring speed of 750 rpm. The calibration solution (1 mM TPP-Cl in 100 mM KCl) was injected automatically with the TIP2k. A calibration range of 0.1-10 µM TPP^+ was covered in 17 steps at 5 min intervals, using data recording intervals of 1 s. 10 calibration runs were performed with each electrode at intervals of 7-14 days. After each run, 5 electrodes were rinsed using the standard procedure (ethanol group) and 5 electrodes were rinsed with water only (water group). The most important quality criteria of an ISE is the sensitivity, defined as the slope of the linear regression of the non-amplified signal (raw signal [mV]) as a function of log[TPP^+], and expressed in mV/decade [mV/dec]. Additional criteria are the raw signal measured at 0 TPP^+ and the response time. The response time was defined as the time after which the slope of the raw signal achieved the value -0.004 mV/s (IUPAC recommendation [1]), measured at the calibration step from 0.8 to 1 µM TPP^+. The number of data points for time derivation was 10. Data shown in Tables 1 and 2 are means ± SD calculated from non-amplified signals. Statistical significance of differences between groups and runs was evaluated using Student´s t-test on log-transformed data.

Calibration test	Rinsing group	Raw signal @ 0 TPP^+ [mV]	Sensitivity @ 1-3 µM TPP^+ [mV/dec]	Critical [TPP^+] @ 40 mV/dec [µM]	|Drift| of raw signal [mV/s]	Response time [s]
1st	Ethanol (5)	-199 ± 10	51 ± 4	0.9 ± 0.5	0.0007	14 ± 3
	Water (5)	-213 ± 18 (3)	52 ± 4	1.0 ± 0.4	0.0006	16 ± 6
5th	Ethanol (5)	-200 ± 14	56 ± 6	0.6 ± 0.3	0.0004	49 ± 10*#
	Water (5)	-199 ± 8	54 ± 3	0.7 ± 0.2	0.0002	28 ± 11
10th	Ethanol (4)	-189 ± 18	54 ± 7	0.9 ± 0.4	0.0006	52 ± 19*
	Water (4)	-198 ± 12	55 ± 5	0.4 ± 0.2*	0.0003	36 ± 14*

Table 2: Performance parameters of TPP^+ electrodes in the course of the study. Ethanol: electrodes rinsed with water, 99% ethanol, water. Water: electrodes rinsed with water. Numbers in brackets show number of electrodes in the group. *P<0.05 vs. 1st run in the same group, #P<0.05 vs. water group in the same run.

Oroboros TPP^+ electrodes are characterized by a high sensitivity (>40 mV/dec for [TPP^+] >0.7 µM) as a basis for extending the limit of detection to <0.2 µM TPP^+ (Table 1). The zero signal, sensitivity, and critical [TPP^+] did not change significantly within 3.5 months (Table 2). The response time increased slightly in the course of the study. There is a trend of a faster increase in response times of electrodes treated with the standard rinsing procedure (including 99% ethanol) compared to rinsing with water only (Table 2).

(1) Improvements of performance parameters of the Oroboros TPP^+ electrode are described here, as a basis for application in mitochondrial physiology with high-resolution respirometry. (2) The low detection limit allows for using low [TPP^+] to avoid inhibition of mitochondrial respiration. (3) Rinsing with ethanol is possible, the same membrane can be used on a TPP^+ electrode for many experiments, and membranes can be exchanged at intervals to ensure high performance.

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1. Buck RP, Lindner E (1994) Recommendation for nomenclature of ion-selective electrodes (IUPAC recommendations 1994). Pure Appl Chem 66: 2527-36.