



## O2k-Protocols

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# Oxygraph-2k Manual Titrations: Mitochondria, Permeabilized Cells, and Biopsies



**OROBOROS INSTRUMENTS Corp**, high-resolution respirometry  
Schöpfstr 18, A-6020 Innsbruck, Austria  
[erich.gnaiger@oroboros.at](mailto:erich.gnaiger@oroboros.at); [www.oroboros.at](http://www.oroboros.at)

### Oxygraph-2k Chamber Volume: 2.0 ml

Substrates	Event	Concentration in syringe (solvent)	Storage [°C]	Final conc. in 2 ml O2k-chamber	Titration [µl] into 2 ml	Syringe [µl]
Pyruvate	<b>P</b>	2 M (H <sub>2</sub> O)	fresh	5 mM	5	25
Malate	<b>M</b>	0.8 M (H <sub>2</sub> O)	-20	2 mM	5	25
Glutamate	<b>G</b>	2 M (H <sub>2</sub> O)	-20	10 mM	10	25
Succinate	<b>S</b>	1 M (H <sub>2</sub> O)	-20	10 mM	20	50
Ascorbate	<b>As</b>	0.8 M (H <sub>2</sub> O)	-20	2 mM	5	25
TMPD	<b>Tm</b>	0.2 M (H <sub>2</sub> O)	-20	0.5 mM	5	25
Cyt. C	<b>c</b>	4 mM (H <sub>2</sub> O)	-20	10 µM	5	25
ADP+ Mg <sup>2+</sup>	<b>D</b>	0.5 M (H <sub>2</sub> O)	-80	1 - 5 mM	4 - 20	10
ATP+ Mg <sup>2+</sup>	<b>T</b>	0.5 M (H <sub>2</sub> O)	-80	1 - 5 mM	4 - 20	10
<b>Uncoupler</b>						
FCCP <sup>1</sup>	<b>F</b>	1 mM (EtOH)	-20	0.5 µM steps	1 µl steps	10
FCCP-MiR06	<b>F</b>	0.1 mM (EtOH)	-20	0.05 µM steps	1 µl steps	10
<b>Inhibitors</b>						
Rotenone	<b>Rot</b>	1.0 or 0.2 mM (EtOH)	-20	0.5 or 0.1 µM	1	10
Malonic acid	<b>Mna</b>	2 M (H <sub>2</sub> O)	fresh	5 mM	5	25
Antimycin A	<b>Ama</b>	5 mM (EtOH)	-20	2.5 µM	1	10
Myxothiazol	<b>Myx</b>	1 mM (EtOH)	-20	0.5 µM	1	10
Sodium azide	<b>Azd</b>	4 M (H <sub>2</sub> O)	-20	≥100 mM	≥50	50
KCN	<b>Kcn</b>	1 M (H <sub>2</sub> O)	fresh	1.0 mM	2	10
Oligomycin	<b>Omy</b>	4 mg/ml (EtOH)	-20	2 µg/ml	1	10
Atractyloside	<b>Atr</b>	50 mM (H <sub>2</sub> O)	-20	0.75 mM	30	50
<b>Cell perm.</b>						
Digitonin	<b>Dig</b>	10 mg/ml (DMSO)	-20	10 µg · 10 <sup>-6</sup> cells	1µl 10 <sup>-6</sup> cells	10
<b>Other</b>						
Catalase	<b>Ctl</b>	112,000 IU/ml (MiR05)	-20	280 IU/ml	5	25
Ionomycin	<b>lmy</b>	0.5 mg/ml (EtOH)	-20	0.5 µg/ml	2	10

<sup>1</sup>Higher FCCP concentration: in various culture media (e.g. RPMI, DMEM) and for TIP2k.