



## O2k-Protocols

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# Oxygraph-2k manual titrations: SUIT protocols with mitochondrial preparations



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### Oxygraph-2k chamber volume: 2.0 ml

Substrates	Event	Concentration in syringe (solvent)	Storage [°C]	Final conc. in 2 ml	Titration [μl]	Syringe [μl]
Pyruvate	P	2 M (H <sub>2</sub> O)	fresh	5 mM	5	25
Malate	M	0.4 M (H <sub>2</sub> O)	-20	0.5 mM	2.5	25
Glutamate	G	2 M (H <sub>2</sub> O)	-20	10 mM	10	25
Succinate	S	1 M (H <sub>2</sub> O)	-20	10 mM	20	50
Octanoyl carnitine	Oct	0.1 M (H <sub>2</sub> O)	-20	0.2 mM	4	10
Ascorbate	As	0.8 M (H <sub>2</sub> O)	-20	2 mM	5	25
TMPD	Tm	0.2 M (H <sub>2</sub> O)	-20	0.5 mM	5	25
Cyt. c	c	4 mM (H <sub>2</sub> O)	-20	10 μM	5	25
ADP+ Mg <sup>2+</sup>	D	0.5 M (H <sub>2</sub> O)	-80	1 - 5 mM	4 - 20	25
ATP+ Mg <sup>2+</sup>	T	0.5 M (H <sub>2</sub> O)	-80	1 - 5 mM	4 - 20	25
<b>Uncoupler</b>						
CCCP*	U	0.1 mM (EtOH)	-20	0.05 μM steps	1 μl steps	10
CCCP*	U	1.0 mM (EtOH)	-20	0.5 μM steps	1 μl steps	10
<b>Inhibitors</b>						
Rotenone	Rot	1 mM (EtOH)	-20	0.5 μM	1	10
Malonic acid	Mna	2 M (H <sub>2</sub> O)	fresh	5 mM	5	25
Antimycin A	Ama	5 mM (EtOH)	-20	2.5 μM	1	10
Myxothiazol	Myx	1 mM (EtOH)	-20	0.5 μM	1	10
Sodium azide	Azd	4 M (H <sub>2</sub> O)	-20	≥100 mM	≥50	50
KCN	Kcn	1 M (H <sub>2</sub> O)	fresh	1.0 mM	2	10
Oligomycin	Omy	5 mM (EtOH)	-20	2.5 μM	1	10
Carboxyatractyloside	Cat	5 mM (H <sub>2</sub> O)	-20	0.75 mM	2	10
<b>Cell perm.</b>						
Digitonin	Dig	10 mg/ml (DMSO)	-20	10 μg·10 <sup>-6</sup> cells	1 μl 10 <sup>-6</sup>	10
<b>Other</b>						
Catalase in MiR06	Ctl	112,000 U/ml	-20	280 U/ml	5	25

\* 0.1 mM stock for mt-preparations with high uncoupler sensitivity; 1 mM stock for mt-preparations with low uncoupler sensitivity, intact cells in various culture media (e.g. RPMI, DMEM, EGC) and for TIP2k.

**Reference:** Pesta D, Gnaiger E (2012) High-resolution respirometry. OXPHOS protocols for human cells and permeabilized fibres from small biopsies of human muscle. *Methods Mol Biol* 810: 25-58. »