



O2k-Protocols

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

OROBOROS INSTRUMENTS, high-resolution respirometry
Schöpfstr 18, A-6020 Innsbruck, Austria
erich.gnaiger@oroboros.at; www.oroboros.at



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 ₂ O)	fresh	5 mM	5	25
Malate	M	0.8 M (H ₂ O)	-20	2 mM	5	25
Glutamate	G	2 M (H ₂ O)	-20	10 mM	10	25
Succinate	S	1 M (H ₂ O)	-20	10 mM	20	50
Octanoyl carnitine	Oct	0.1 M (H ₂ O)	-20	0.2 mM	4	10
Ascorbate	As	0.8 M (H ₂ O)	-20	2 mM	5	25
TMPD	Tm	0.2 M (H ₂ O)	-20	0.5 mM	5	25
Cyt. c	c	4 mM (H ₂ O)	-20	10 μM	5	25
ADP+ Mg ²⁺	D	0.5 M (H ₂ O)	-80	1 - 5 mM	4 - 20	25
ATP+ Mg ²⁺	T	0.5 M (H ₂ O)	-80	1 - 5 mM	4 - 20	25
Uncoupler						
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
Inhibitors						
Rotenone	Rot	1 mM (EtOH)	-20	0.5 μM	1	10
Malonic acid	Mna	2 M (H ₂ 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 ₂ O)	-20	≥100 mM	≥50	50
KCN	Kcn	1 M (H ₂ O)	fresh	1.0 mM	2	10
Oligomycin	Omy	5 mM (EtOH)	-20	2.5 μM	1	10
Carboxyatractyloside	Cat	5 mM (H ₂ O)	-20	5 μM	2	10
Other						
Digitonin	Dig	10 mg/ml (DMSO)	-20	10 μg · 10 ⁻⁶ cells	1 μl 10 ⁻⁶	10
Catalase in MiR06	Ctl	112,000 U/ml	-20	280 U/ml	5	25
Hydrogen peroxide for reoxygenation	Hp	200 mM	fresh		1 - 3	10

* 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. »[Bioblast Access](http://www.bioblast.at)

Oxygraph-2k chamber volume: 2.0 ml

Fluorescence probes and related	Event	Concentration in syringe (solvent)	Storage [°C]	Final conc. in 2 ml	Titration [µl]	Syringe [µl]
Amplex®UltraRed	AmR	10 mM (DMSO)	-20	10 µM	2	10
Horse radish peroxidase	HRP	500 U/ml (MiR05)	-20	1 U/ml	4	10
Superoxide dismutase	SOD	check supplier information	4-8	5 U/ml		10
Hydrogen peroxide for calibration	Hp	0.04 mM (H ₂ O)	fresh	0.1 µM	5	10
Safranin	Saf	0.2 mM (H ₂ O)	-20	0.25 µM	2.5	10
TMRM	Tmr	0.2 mM (H ₂ O)	-20	0.25 µM	2.5	10

Reference: Krumschnabel G, Eigentler A, Fasching M, Gnaiger E (2014) Use of safranin for the assessment of mitochondrial membrane potential by high-resolution respirometry and fluorometry. *Methods Enzymol* 542:163-81. »[Bioblast Access](#)

Krumschnabel G, Fontana-Ayoub M, Sumbalova Z, Heidler J, Gauper K, Fasching M, Gnaiger E (2015) Simultaneous high-resolution measurement of mitochondrial respiration and hydrogen peroxide production. *Methods Mol Biol* 1264:245-61. »[Bioblast Access](#)