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Updates: http://wiki.oroboros.at/index.php/MiPNet09.12_O2k-Titrations

O2k manual titrations: SUIT protocols with mitochondrial preparations

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O2k-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_2O)	fresh	5 mM	5	25
Malate	M	0.4 M (H_2O)	-20	2 mM	10	25
Malate[1]	M	0.05 M (H_2O)	-20	0.1 mM	4	10
Glutamate	G	2 M (H_2O)	-20	10 mM	10	25
Succinate[2]	S	1 M (H_2O)	-20	10 mM	20	50
Octanoylcarnitine	Oct	0.1 M (H_2O or DMSO)	-20	0.5 mM	10	25
Ascorbate	As	0.8 M (H_2O)	-20	2 mM	5	25
TMPD	Tm	0.2 M (H_2O)	-20	0.5 mM	5	25
Cyt. *c*	c	4 mM (H_2O)	-20	10 µM	5	25
ADP+ Mg^{2+}	D	0.5 M (H_2O)	-80	1-5 mM	4-20	25
ATP+ Mg^{2+}	T	0.5 M (H_2O)	-80	1-5 mM	4-20	25
Glucose	Glc	2 M (H_2O)	fresh	20 mM	20	50
Glycerophosphate	Gp	1 M (H_2O)	-20	10 mM	20	50
Uncoupler						
CCCP[3]	U	0.1 mM (EtOH)	-20	0.05 µM steps	1 µL steps	10
CCCP[3]	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_2O)	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_2O)	-20	≥100 mM	≥50	100
KCN	KCN	20 mM (H_2O)	-20	1 mM	100	100
Oligomycin[4]	Omy	0.01 mM (EtOH)	-20	5-10 nM	1-2	10
Carboxyatractyloside	Cat	2 mM (H_2O)	-20	1-5 µM	1-2	10
Salicylhydroxamic acid	SHAM	20 mM (DMSO)	-20	1 mM	100	100
Other						
Digitonin[5]	Dig	10 mg/mL (DMSO)	-20	5 µg/mL	1	10
Catalase in MiR06	Ctl	112,000 U/mL	-20	280 U/mL	5	25
Hydrogen peroxide (for reoxygenation)	H2O2	200 mM	fresh		1-3	10

[1] Low concentration of M (typically 0.1 mM) does not saturate the N-pathway, but saturates the F-pathway.
[2] The concentration of S may be increased up to 50 mM after Rot to compensate for the inhibitory effect of M.
[3] 0.1 mM stock for mt-preparations with high uncoupler sensitivity; 1 mM stock for mt-preparations with low uncoupler sensitivity, living cells in various culture media (*e.g.*, RPMI, DMEM, EGC) and for TIP2k.
[4] Omy (2.5 µM final conc.) displays a strong inhibitory effect on *E* in various sample preparations; therefore, diluted Omy must be tested in each sample preparation.
[5] The optimum effective Dig concentration for complete plasma membrane permeabilization of cultured cells can be determined directly in a respirometric protocol (see DL-Protocol: SUIT-010 O2 ce-pce D008).

O2k-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]
DTPA	DTPA	5 mM (H_2O)	-20	15 µM	6	10
Amplex®UltraRed	AmR	10 mM (DMSO)	-20	10 µM	2	10
Horseradish 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)	H2O2	0.04 mM (H_2O)	fresh	0.1 µM	5	10
Safranin	Saf	0.5 mM (H_2O)	RT	0.5 µM	2	10
TMRM	TMRM	0.2 mM (DMSO)	-20	0.2 µM	2	10
Rhodamine 123	Rh123	0.2 mM (EtOH)	-20	0.2 µM	2	10
Calcium Green	CaG	2 mM (H_2O)	-20	1 µM	1	10
Magnesium Green	MgG	1.1 mM (H_2O)	-20	1.1 µM	2	10
Coenzyme Q_2	Q2	10 mM (EtOH)	-20	30 µM	6	10
Coenzyme Q_2	Q2	1 mM (EtOH)	-20	1 µM	2	10

Further abbreviations

Atractyloside	Atr
Calcium	Ca^{2+}
Dinitrophenol	DNP; U
Diethylentriamin-N,N,N',N,N-pentaacetic acid	DTPA
Carbonyl cyanide p-trifluoromethoxyphenyl hydrazone	FCCP; U
Hydroxycinnamate	Hci
Oxaolacetate	Oa
Octanoate	Oca; FA
Palmitate	Paa; FA
Palmitoylcarnitine	Pal; FA
Tetraphenylphosphonium ion	TPP^+

References

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O2k-Chamber volume: 0.5 mL (O2k-sV-Module)

Substrates	Event	Concentration in syringe (solvent)	Storage [°C]	Final conc. in 0.5 mL	Titration [µL]	Syringe [µL]
Pyruvate	P	2.5 M (H_2O)	fresh	5 mM	1	10
Malate	M	0.4 M (H_2O)	-20	2 mM	2.5	10
Malate[1]	M	0.05 M (H_2O)	-20	0.1 mM	1	10
Glutamate	G	2 M (H_2O)	-20	10 mM	2.5	10
Succinate[2]	S	1 M (H_2O)	-20	10 mM	5	25
Octanoylcarnitine	Oct	0.1 M (H_2O or DMSO)	-20	0.5 mM	2.5	10
Ascorbate	As	1 M (H_2O)	-20	2 mM	1	10
TMPD	Tm	0.25 M (H_2O)	-20	0.5 mM	1	10
Cyt. *c*	c	5 mM (H_2O)	-20	10 µM	1	10
ADP+ Mg^{2+}	D	0.5 M (H_2O)	-80	1-5 mM	1-5	10
ATP+ Mg^{2+}	T	0.5 M (H_2O)	-80	1-5 mM	1-5	10
Glucose	Glc	2 M (H_2O)	fresh	20 mM	5	25
Glycerophosphate	Gp	1 M (H_2O)	-20	10 mM	5	25
Uncoupler						
CCCP[3]	U	0.025 mM (EtOH)	-20	0.05 µM steps	1 µL steps	10
CCCP[3]	U	0.25 mM (EtOH)	-20	0.5 µM steps	1 µL steps	10
Inhibitors						
Rotenone	Rot	0.25 mM (EtOH)	-20	0.5 µM	1	10
Malonic acid	Mna	0.5 M (H_2O)	fresh	5 mM	5	25
Antimycin A	Ama	1.25 mM (EtOH)	-20	2.5 µM	1	10
Myxothiazol	Myx	0.25 mM (EtOH)	-20	0.5 µM	1	10
Sodium azide	Azd	2 M (H_2O)	-20	≥100 mM	≥25	100
KCN	KCN	0.25 M (H_2O)	-20	1 mM	2	10
Oligomycin[4]	Omy	2.5 µM (EtOH)	-20	5-10 nM	1-2	10
Carboxyatractyloside	Cat	0.5 mM (H_2O)	-20	1-5 µM	1-5	10
Other						
Digitonin[5]	Dig	2.5 mg/mL (DMSO)	-20	5 µg/mL	1	10
Catalase in MiR06	Ctl	28,000 U/mL	-20	280 U/mL	5	25
Hydrogen peroxide (for reoxygenation)	H2O2	50 mM	fresh		1-3	10

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Oxaolacetate	Oa
Octanoate	Oca; FA
Palmitate	Paa; FA
Palmitoylcarnitine	Pal; FA

References

Doerrier C, Garcia-Souza LF, Krumschnabel G, Wohlfarter Y, Mészáros AT, Gnaiger E (2018) High-Resolution FluoRespirometry and OXPHOS protocols for human cells, permeabilized fibers from small biopsies of muscle, and isolated mitochondria. Methods Mol Biol 1782:31-70. »Bioblast link«

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