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O2k manual titrations: SUIT protocols with mitochondrial preparations

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

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	0.8 M (H_2O)	-20	2 mM	5	25
Glutamate	G	2 M (H_2O)	-20	10 mM	10	25
Succinate*	S	1 M (H_2O)	-20	10 mM	20	50
Octanoyl carnitine	Oct	0.1 M (H_2O)	-20	0.2 mM	4	10
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
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_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	**50**
KCN	Kcn	1 M (H_2O)	fresh	1.0 mM	2	**10**
Oligomycin	Omy	5 mM (EtOH)	-20	2.5 µM	1	**10**
Carboxyatractyloside	Cat	5 mM (H_2O)	-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

* The oncentration of S may be increased up to 50 mM after Rot to compensate for the inhibitory effect of M.
‡ 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.

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]
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_2O)	fresh	0.1 µM	5	10
Safranin	Saf	0.2 mM (H_2O)	RT	0.25 µM	2.5	10
TMRM	Tmr	0.2 mM (H_2O)	-20	0.25 µM	2.5	10
Calcium Green	CaG	2 mM (H_2O)	-20	1 µM	1	10
Magnesium Green	MG	5 mM (H_2O)	-20	2.5 µM	1	10

Further abbreviations

Atractyloside	Atr
Calcium	Ca^{2+}
Dinitrophenol	DNP; U
Carbonyl cyanide p-trifluoro-methoxyphenyl hydrazone	FCCP; U
Glucose	Glc
Glycerophosphate	Gp
Hydroxycinnamate	Hci
Oxaolacetate	Oa
Octanoate	Oca; FA
Palmitate	Paa; FA
Palmitoylcarnitine	Pal; FA
Tetraphenyl-phosphonium ion	TPP^+

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