

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 ₂ O)	fresh	5 mM	5	25
Malate	M	0.4 M (H ₂ O)	-20	2 mM	10	25
Malate	M	0.05 M (H ₂ O)	-20	0.1 mM	4	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	0.01 mM (EtOH)	-20	5-10 nM	1-2	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	H ₂ O ₂	200 mM	fresh		1 - 3	10

for reoxygenation						
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* The concentration 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.

** Oligomycin (2.5 μ M final conc.) displays a strong inhibitory effect on *E* in various sample preparations; therefore, diluted Omy must be tested and used.

O2k-Chamber volume: 2.0 mL

Fluorescence probes and related	Event	Concentration in syringe (solvent)	Storage [°C]	Final conc. in 2 mL	Titration [μ L]	Syringe [μ L]
DTPA	DTPA	5 mM (H ₂ O)	-20	15 μ M	6	25
Amplex®UltraRed	AmR	10 mM (DMSO)	-20	10 μ M	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	H ₂ O ₂	0.04 mM (H ₂ O)	fresh	0.1 μ M	5	10
Safranin	Saf	0.2 mM (H ₂ O)	RT	0.25 μ M	2.5	10
TMRM	TMRM	0.2 mM (H ₂ O)	-20	0.25 μ M	2.5	10
Calcium Green	CaG	2 mM (H ₂ O)	-20	1 μ M	1	10
Magnesium Green	MgG	5 mM (H ₂ O)	-20	2.5 μ M	1	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
Glucose	Glc
Glycerophosphate	Gp
Hydroxycinnamate	Hci
Oxaolacetate	Oa
Octanoate	Oca; FA
Palmitate	Paa; FA
Palmitoylcarnitine	Pal; FA
Tetraphenylphosphonium ion	TPP ⁺

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