

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 ¹	M	0.05 M (H ₂ O)	-20	0.1 mM	4	10
Glutamate	G	2 M (H ₂ O)	-20	10 mM	10	25
Succinate ²	S	1 M (H ₂ O)	-20	10 mM	20	50
Octanoylcarnitine	Oct	0.1 M (H ₂ O)	-20	0.5 mM	10	25
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
Glucose	Glc	2 M (H ₂ O)	fresh	20 mM	20	50
Glycerophosphate	Gp	1 M (H ₂ O)	-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 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	100
KCN	KCN	20 mM (H ₂ O)	-20	1 mM	100	100
Oligomycin ⁴	Omy	0.01 mM (EtOH)	-20	5-10 nM	1-2	10
Carboxyatractyloside	Cat	2 mM (H ₂ O)	-20	1-5 µM	1-5	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 μ M 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 [μ L]	Syringe [μ L]
DTPA	DTPA	5 mM (H ₂ O)	-20	15 μ M	6	10
Amplex@UltraRed	AmR	10 mM (DMSO)	-20	10 μ M	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 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
Rhodamine 123	Rh123	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
Coenzyme Q ₂	Q2	10 mM (EtOH)	-20	30 μ M	6	10
Coenzyme Q ₂	Q2	1 mM (EtOH)	-20	1 μ M	2	10

Further abbreviations

Atractyloside	Atr
Calcium	Ca ²⁺
Dinitrophenol	DNP; U
Diethyltriamin-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

- Chinopoulos C, Kiss G, Kawamata H, Starkov AA (2014) Measurement of ADP-ATP exchange in relation to mitochondrial transmembrane potential and oxygen consumption. *Methods Enzymol* 542:333-48. »[Bioblast link](#)«
- Doerrier C, Garcia-Souza LF, Krumschnabel G, Wohlfarter Y, Mészáros AT, Gnaiger E (2018) High-Resolution Fluorescence Respirometry and OXPHOS protocols for human cells, permeabilized fibers from small biopsies of muscle, and isolated mitochondria. *Methods Mol Biol* 1782:31-70. »[Bioblast link](#)«
- Elustondo PA, Negoda A, Kane CL, Kane DA, Pavlov EV (2014) Spermine selectively inhibits high-conductance, but not low-conductance calcium-induced permeability transition pore. *Biochim Biophys Acta* 1847:231-40. »[Bioblast link](#)«
- Gnaiger E (2014) Mitochondrial pathways and respiratory control. An introduction to OXPHOS analysis. 4th ed. *Mitochondr Physiol Network* 19.12. Oroboros MiPNet Publications, Innsbruck:80 pp. »[Bioblast link](#)«
- 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 link](#)«
- 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 link](#)«

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 ₂ O)	fresh	5 mM	1	10
Malate	M	0.4 M (H ₂ O)	-20	2 mM	2.5	10
Malate ¹	M	0.05 M (H ₂ O)	-20	0.1 mM	1	10
Glutamate	G	2 M (H ₂ O)	-20	10 mM	2.5	10
Succinate ²	S	1 M (H ₂ O)	-20	10 mM	5	25
Octanoylcarnitine	Oct	0.1 M (H ₂ O)	-20	0.5 mM	2.5	10
Ascorbate	As	1 M (H ₂ O)	-20	2 mM	1	10
TMPD	Tm	0.25 M (H ₂ O)	-20	0.5 mM	1	10
Cyt. c	c	5 mM (H ₂ O)	-20	10 μM	1	10
ADP+ Mg ²⁺	D	0.5 M (H ₂ O)	-80	1-5 mM	1-5	10
ATP+ Mg ²⁺	T	0.5 M (H ₂ O)	-80	1-5 mM	1-5	10
Glucose	Glc	2 M (H ₂ O)	fresh	20 mM	5	25
Glycerophosphate	Gp	1 M (H ₂ O)	-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 ₂ O)	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 ₂ O)	-20	≥100 mM	≥25	100
KCN	KCN	0.25 M (H ₂ O)	-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 ₂ O)	-20	1-5 μM	1-5	10
Salicylhydroxamic acid	SHAM	0.25 M (DMSO)	-20	1 mM	2	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	10
Hydrogen peroxide (for reoxygenation)	H2O2	50 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.

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