

# 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 <sub>2</sub> O)	fresh	5 mM	5	25
Malate	M	0.4 M (H <sub>2</sub> O)	-20	2 mM	10	25
Malate <sup>1</sup>	M	0.05 M (H <sub>2</sub> O)	-20	0.1 mM	4	10
Glutamate	G	2 M (H <sub>2</sub> O)	-20	10 mM	10	25
Succinate <sup>2</sup>	S	1 M (H <sub>2</sub> O)	-20	10 mM	20	50
Octanoylcarnitine	Oct	0.1 M (H <sub>2</sub> O)	-20	0.5 mM	10	25
Ascorbate	As	0.8 M (H <sub>2</sub> O)	-20	2 mM	5	25
TMPD	Tm	0.2 M (H <sub>2</sub> O)	-20	0.5 mM	5	25
Cyt. c	c	4 mM (H <sub>2</sub> O)	-20	10 μM	5	25
ADP+ Mg <sup>2+</sup>	D	0.5 M (H <sub>2</sub> O)	-80	1-5 mM	4-20	25
ATP+ Mg <sup>2+</sup>	T	0.5 M (H <sub>2</sub> O)	-80	1-5 mM	4-20	25
Glucose	Glc	2 M (H <sub>2</sub> O)	fresh	20 mM	20	50
Glycerophosphate	Gp	1 M (H <sub>2</sub> O)	-20	10 mM	20	50
<b>Uncoupler</b>						
CCCP <sup>3</sup>	U	0.1 mM (EtOH)	-20	0.05 μM steps	1 μL steps	10
CCCP <sup>3</sup>	U	1.0 mM (EtOH)	-20	0.5 μM steps	1 μL steps	10
<b>Inhibitors</b>						
Rotenone	Rot	1 mM (EtOH)	-20	0.5 μM	1	10
Malonic acid	Mna	2 M (H <sub>2</sub> 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 <sub>2</sub> O)	-20	≥100 mM	≥50	100
KCN	KCN	20 mM (H <sub>2</sub> O)	-20	1 mM	100	100
Oligomycin <sup>4</sup>	Omy	0.01 mM (EtOH)	-20	5-10 nM	1-2	10
Carboxyatractyloside	Cat	2 mM (H <sub>2</sub> O)	-20	1-5 μM	1-5	10
Salicylhydroxamic acid	SHAM	20 mM (DMSO)	-20	1 mM	100	100
<b>Other</b>						
Digitonin <sup>5</sup>	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

<sup>1</sup>Low concentration of M (typically 0.1 mM) does not saturate the N-pathway, but saturates the F-pathway.

<sup>2</sup> The concentration of S may be increased up to 50 mM after Rot to compensate for the inhibitory effect of M.

<sup>3</sup> 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.

<sup>4</sup> 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.

<sup>5</sup> 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 <sub>2</sub> O)	-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 <sub>2</sub> O)	fresh	0.1 µM	5	10
Safranin	Saf	0.2 mM (H <sub>2</sub> O)	RT	0.25 µM	2.5	10
TMRM	TMRM	0.2 mM (H <sub>2</sub> O)	-20	0.25 µM	2.5	10
Rhodamine 123	Rh123	0.2 mM (H <sub>2</sub> O)	-20	0.25 µM	2.5	10
Calcium Green	CaG	2 mM (H <sub>2</sub> O)	-20	1 µM	1	10
Magnesium Green	MgG	5 mM (H <sub>2</sub> O)	-20	2.5 µM	1	10
Coenzyme Q <sub>2</sub>	Q2	10 mM (EtOH)	-20	30 µM	6	10
Coenzyme Q <sub>2</sub>	Q2	1 mM (EtOH)	-20	1 µM	2	10

## Further abbreviations

Atractyloside	Atr
Calcium	Ca <sup>2+</sup>
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 <sup>+</sup>

## References

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