Mitochondrial respiration and ATP production [1, 2] on homogenate of red muscle of Sea bass

Products:

- **MiR05 buffer** (without EGTA and $MgCl_2$) - aliquots (20*50 mL) at -20°C

	Sigma ref	Store at	Final conc.	FW	Add to 1L
HPLC H_2O					500 mL
K-lactobionate	153516-100G	RT	60mM	358.3	120mL Of 0.5M*
Taurine	T0625-25G	RT	20mM	125.1	2.502g
KH_2PO_4	P9791-1KG	RT	10mM	136.1	1.361g
HEPES	H7523-250G	RT	20mM	238.3	4.77g
Sucrose	S9378-1KG	RT	110mM	342.3	37.65g
BSA[1]	A6003-25G	4 °C	1g/L		1g
[2]KOH (Ca^{2+}free)	P5958-250G	RT	5N	56.11	pH 7.2
HPLC H_2O					Qsp 1L

***K-lactobionate 0.5M** - Freshly prepared

	Vol final = 240 mL	Vol final = 120 mL
Lactobionic acid	43 g	21.50 g
HPLC H_2O	160 mL	80 mL
KOH (Ca^{2+}free)	pH 7.0 at RT	pH 7.0 at RT
HPLC H_2O	Qsp 240 mL	Qsp 120 mL

-Adenylate kinase inhibitor (Ap5A) - aliquots of 100µL at -20°C (**07/04/2019 – Lot SLBT4515**)

	Sigma ref	Store at	Final conc.	FW	1950µL dH_2O
Ap5A	D4022-50mg	-20°C	25mM	1026.28	50mg

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- **Pyruvate** - Freshly prepared

	Sigma ref	Store at	Final conc.	FW	200µL H_2O δ
Sodium pyruvate	P2256-25 g	4°C	2M	110.0	44 mg

- **Malate** –aliquots (10*500 µL) at -20°C

	Sigma ref	Store at	Final conc.	FW	5mL H_2O δ
Malic acid	M1000-100 g	RT	400mM	134.1	268.2mg
HPLC H_2O					3mL
KOH (Ca^{2+}free)		RT	5N	56.11	pH 7.1 at RT (≈ 700 µL)
HPLC H_2O					Qsp 5mL

[1] Preliminary dissolved in heated up H2O δ

[2] No longer sold by Sigma, found under same ref at Honeywell Speciality Chemicals, Germany

- Succinate disodium salt, hexahydrate - aliquots (10*500 *µL)* at -20°C

	Sigma ref	Store at	Final conc.	FW	5mL
Succinate	S2378-100 g	RT	1M	270.1	1.3505g
HPLC H_2O					3mL
TrisHCl	T3253-250 g	RT	1N	157.60	pH 7
HPLC H_2O					Qsp 5mL

- Magnesium Green 5N hexapotassium salt- aliquots (20*50µL; in opaque tubes) at -20°C

	Life technologies	Store at	Final conc.	FW	1mL HPLC H_2O
MgG $5K^+$	M3733-1mg	-20°C	1.1mM	915.9004	1mg

- Magnesium chloride ($MgCl_2$) – Room temperature

	Sigma ref	Store at	Final conc.	FW	10mL HPLC H_2O
$MgCl_2•6H_2O$	M2670-500g	RT	1M	203.30	2.0g

- Dilute to 200mM by adding 2mL of 1M stock to 8mL of HPLC H_2O.

- EDTA – Room temperature

	Sigma ref	Store at	Final conc.	FW	10mL HPLC H_2O
EDTA	E9884-100g	RT	200mM	292.24	585mg
KOH	P5958-250g	RT	5N	56.11	pH 6
		Heat up solution			
KOH	P5958-250g	RT	5N	56.11	pH 7.1

- Dilute to 1mM by adding 50µL of 200mM stock to 9950µL of HPLC H_2O.

- EGTA – Room temperature

	Sigma ref	Store at	Final conc.	FW	10mL HPLC H_2O
EGTA	E4378-100mg	RT	200mM	380.35	761mg
KOH	P5958-250g	RT	5N	56.11	pH 7.1
		Heat up solution			
KOH	P5958-250g	RT	5N	56.11	pH 7.1

- ADP K^+ 250 mM - aliquots (40*200*µL*) at -80°C (<u>NO freeze-thaw</u> cycle)

	Merck ref	Store at	Final conc.	FW	8mL HPLC H_2O
ADP	117105-1G	-20°C	250 mM	501.3	1g
HPLC H_2O					4mL
KOH	P5958-250G	RT	5 N	56.11	pH 6.3-6.9 at RT (~750µL)
HPLC H_2O					Qsp 8 mL

- Carboxylatractyloside potassium salt from *Xanthium sibiricum* – aliquots (11*200µL) at -20°C

	Sigma ref	Store at	Final conc.	FW	2.1mL HPLC H_2O
cATR	216201-2mg	-20°C	1mM	939.1	2mg

- **Antimycin A** (from *Streptomyces sp.*) - aliquots of 200µL at -20°C.

	Sigma ref	Store at	Final conc.	FW	2mL Eth 99%
Antimycin A	A8674-25 mg	-20 °C	5mM	540.0	5.4mg

-Ascorbate sodium salt (Sodium L-ascorbate) – aliquots (20*500µL) in opaque tubes in -20°C – use one a day

	Sigma ref	Store at	Final conc.	FW	10mL HPLC H_2O
Ascorbate	A4034-100 g	RT	800mM	198.11	1.584g

-N,N,N',N'-Tetramethyl-p-phenylenediamine dihydrochloride- aliquots (10*120µL) in opaque tubes at -20°C – use one a day

	Sigma ref	Store at	Final conc.	FW	1.2mL HPLC H_2O
TMPD	T3134-5 g	RT	200mM	237.2	56.9mg

Cleaning:

- *3 times distilled water*
- *2 times Eth 70%*
- *5 min Eth 70%*
- *10 min Eth 100%*
- *5 min Eth 100% ----- or: Overnight Eth 70%*
- *2 times Eth 70%*
- *3 times distilled water*

References:

1. Salin, K., et al., *Variation in metabolic rate among individuals is related to tissue-specific differences in mitochondrial leak respiration.* Physiological and Biochemical Zoology, 2016. **89**(6): p. 511-523.
2. Salin, K., et al., *Simultaneous measurement of mitochondrial respiration and ATP production in tissue homogenates and calculation of effective P/O ratios.* Physiological Reports, 2016. **4**(20): p. e13007.

Methods:

-2.1mL of **red muscle** (1 mg/mL) homogenate in MiR05 buffer (<u>without</u> EGTA and $MgCl_2$).

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-Add 8µL of Ap5A 25mM (Cf = 100µM) with a pipette

----------Put stoppers----------

Chamber _ Oroboros _ → **Add** oxygen the medium (550 µM < $[O_2]_{13°C}$ < 650 µM)

----------OR--------

Chamber _ Oroboros _ → **Add** oxygen the medium (550 µM < $[O_2]_{16°C}$ < 650 µM)

----------OR--------

Chamber _ Oroboros _ → **NO** oxygen the medium ($[O_2]_{16°C}$ = 276 µM)

----------OR--------

Chamber _ Oroboros _ → **NO** oxygen the medium ($[O_2]_{13°C}$ = 295 µM)

----------*Close chamber*----------

- Add 5µL of **Pyruvate** 2M (Cf = 5mM)
- Add 2.5µL of **Malate** 400mM (Cf = 0.5mM)

- Add 4µL of **MgGreen** 1.1mM (Cf = 2.2µM)

----------*Reading for 30sec*----------

-Add 1µL of **EGTA** 200mM (Cf = 0.1mM)

----------*Reading for* 30sec ----------

-Add 10µL of **EDTA** 1mM (Cf = 5µM)

----------*Reading for* 30sec ----------

REPEAT PREVIOUS STEP 2 MORE TIMES [EDTA (Cf = 15µM)]

-Add 1µL of **$MgCl_2$** 200mM (Cf = 0.1mM)

----------*Reading for* 30sec ----------

REPEAT PREVIOUS STEP 9 MORE TIMES [MgCl2 (Cf = 1mM)]

----------*Reading for* 30sec ----------

-Add 20µL of **Succinate** 1M (Cf = 10µM)

----------*Reading for 5 minutes*----------

-Add 16µL of **ADP** 250mM (Cf = 2mM)

---------- *Reading until stabilization* ----------

-Add 8µL of **cATR** 1mM (Cf = 4µM)

----------*Reading until stabilization*----------

- Add 1µL of **Antimycin A** 5mM (Cf = 2.5µM)

----------*Reading until stabilization*----------

- Add 20µL of **Ascorbate** 800mM (Cf = 8mM)

----------*Reading until stabilization*----------

- Add 5µL of **TMPD** 200mM (Cf = 500µM)

----------*Reading 5 minutes*----------