

## **Respiration Buffers for Skinned Fibers**

### **-20 C Liquid Stocks:**

(see Table for Correct Product Catalog Numbers)

#### 100 mM DTT

Take 0.77g in 50ml of ddH<sub>2</sub>O. Make aliquots of 1ml and store at -20

#### 200 mM ADP

Take 0.17g in 2ml ddH<sub>2</sub>O. Make aliquots of 50ul and store at -20  
Need to add 10ul/2ml of respiration buffer to achieve state 3

#### 1.6 mM Cytochrome C

Take 0.039g in 2ml of Buffer C, Make aliquots of 50ul and store at -20  
Need to add 10ul/2ml of respiration buffer

#### 200 ug/ml oligomycin

Take 0.005g in 12.5ml of ddH<sub>2</sub>O and 12.5ml 100% ethanol (routinely used)  
(alternatively, dissolve in 25ml of 100% ethanol)  
Make aliquots of 1ml and store at -20  
Need to add 10ul/2ml of respiration buffer to achieve state 4

#### 50 mg/ml Saponin

Take 0.05g in 1ml of ddH<sub>2</sub>O. Make aliquots of 20ul and store at -20  
Need to add 3 uL/3mL of Buffer A at time of permeabilization.

#### 8 mM Palmitoyl-L-Carnitine

Take 10mg vial and dilute with 2941ul ddH<sub>2</sub>O  
Make aliquots of 120ul and store at -20  
Need to add 50ul/2ml of respiration buffer.

### **Stock Made on Day of Experiment (if using succinate/rotenone):**

#### 10uM Rotenone

Dissolve 3.9mg rotenone in 1ml DMSO  
Add 10ul per 10ml respiration buffer  
(make rotenone solution on day of experiment)

**+4 C Liquid Stocks Remade Monthly:**

100mM K<sub>2</sub>CaEGTA (heated to dissolve) store at 4C for a month

EGTA            3.8g  
KOH             1.12g  
CaCO<sub>3</sub>         1g  
QS to 100ml

100mM K<sub>2</sub>EGTA (heated to dissolve) store at 4C for a month

EGTA            3.8g  
KOH             1.12  
QS to 100ml

**Must REHEAT before each use since routinely precipitates.**

100mM MgCl<sub>2</sub> store at 4C for a month

2.03g in 100mls

100mM Imidazole store at 4C for a month

0.68g in 100mls

100mM KH<sub>2</sub>PO<sub>4</sub> store at 4C for a month

1.36g in 100ml

### Actual Respiration Buffers:

Can weigh dry reagents up to one week before and keep frozen (-20 C, wrapped in parafilm and aluminum foil). (Weights of reagents for given volume of buffer are expressed in grams below.)

#### **Buffer A (pH=7.1 at 25C) pH with Glacial Acetic Acid (Fisher A38-212)**

<b>Product</b>	<b>mM</b>	<b>20ml</b>	<b>40ml</b>	<b>60ml</b>
K <sub>2</sub> CaEGTA (ml)	2.77	0.554	1.108	1.662
K <sub>2</sub> EGTA (ml)	7.23	1.446	2.892	4.338
MgCl <sub>2</sub> (ml)	6.56	1.312	2.624	3.936
Imidazole (ml)	20	4	8	12
K-MES (g)	53.3	.2487	0.4974	.74609
Taurine (g)	20	.05004	0.10008	0.15012
ATP (g)	5.3	.05842	0.11683	0.17525
PCr (g)	15	.07653	0.15306	0.22959
KH <sub>2</sub> PO <sub>4</sub> (ml)	3	0.6	1.2	1.8
DTT (ml)	0.5	0.1	0.2	0.3

#### **Buffer B (pH=7.1 at 25C), pH with Glacial Acetic Acid (Fisher A38-212)**

<b>Product</b>	<b>mM</b>	<b>20ml</b>	<b>40ml</b>	<b>60ml</b>
K <sub>2</sub> CaEGTA (ml)	2.77	0.554	1.108	1.662
K <sub>2</sub> EGTA (ml)	7.23	1.446	2.892	4.338
MgCl <sub>2</sub> (ml)	1.38	0.276	0.552	0.828
Imidazole (ml)	20	4	8	12
K-MES (g)	100	0.4666	.9332	1.3998
Taurine (g)	20	0.05004	0.10008	0.15012
KH <sub>2</sub> PO <sub>4</sub> (ml)	3	0.6	1.2	1.8
DTT (ml)	0.5	0.1	0.2	0.3

#### **Add the same day as experiment**

<b>BSA (g)</b>	2mg/ml	0.04	0.08	.12
----------------	--------	------	------	-----

#### **Buffer C (KCL Buffer) pH=7.1 at 25C**

**pH adjusted with 1 M KOH**

**(made from KOH pellets, Sigma P-5958)**

<b>Product</b>	<b>mM</b>	<b>20ml</b>	<b>40ml</b>	<b>60ml</b>
KCL (g)	125	0.1864	0.3728	0.5592
HEPES (g)	20	0.09532	0.19064	0.28596
Mg-Acetate (g)	3	0.01287	0.02574	0.03861
EGTA (g)	0.4	0.00304	0.00609	0.00913
DTT (ml)	0.3	0.06	0.12	0.18
KH <sub>2</sub> PO <sub>4</sub> (ml)	5	1	2	3

#### **Add the same day as experiment**

<b>BSA (g)</b>	2mg/ml	0.04	0.08	.12
----------------	--------	------	------	-----

## SUBSTRATES

Add the same day as experiment to either Buffer B or C; usually Buffer C is used for respiration chamber since allows for cyt c test.

Generally, for experiments involving 3 substrates, 60 mL of Buffer C will be made from dry reagents for “60 mL of Buffer C” with addition of BSA, bringing initially to 45 mL volume, splitting into three 15 mL aliquots, adding 3 different substrate sets employing 20 mL amounts of substrates below, then pH to 7.1, and QS to 20 mL, finally mixing well. Once substrate-containing buffers completed, place respiration buffers in 50 mL conical vials.

	mM	20	40	60
Glutamate (g)	5mM	0.01471	0.02942	.04413
Malate (g)	2mM	.00712	0.01424	0.02137
<b>glutamate/malate is stirred x 20 min prior to pHing</b>				
Pyruvate (g)	10mM	0.022	0.044	0.066
Malate (g)	5mM	0.01781	0.03561	0.05342
Palmitoyl-carnitine (ul)	20uM	50	100	150
Malate (g)	5mM	0.01781	0.03561	0.05342
Succinate (g)	5mM	0.027	0.054	0.081
Rotenone (ul)	10uM	20	40	60

## Products

Product	FW	Company	Cat. N	Dry Stock Storage/ Dessicator
ADP	427.2	Sigma	A-2754	-20 C / Dessicator
ATP	551.1	Sigma	A-7699	-20 C / Dessicator
BSA		Sigma	A-8022	+ 4 C/ Dessicator
CaCO <sub>3</sub>	100.1	Sigma	C-4830	RT
Creatine	131.1	Sigma	C-3630	RT
Creatine phosphate	255.1	Sigma	P-7936	-20 C / Dessicator
Cytochrome c	12.384	Sigma	C-2506	-20 C / Dessicator
DTT	154.2	Sigma	D-5545	+ 4 C / Dessicator
EGTA	380.4	Sigma	E-3889	RT
Glutamate	147.1	Sigma	G-1001	RT
HEPES	238.3	Sigma	H-7523	RT
Imidazole	68.08	Sigma	I-2399	RT
KCl	74.56	Sigma	P-3911	RT
KH <sub>2</sub> PO <sub>4</sub>	136.09	Fisher	P380-500	RT
K-MES	233.3	Sigma	M-0895	RT
KOH	56.11	Sigma	P-5958	RT
Malate	178.05	Sigma	M-6773	RT
MgAcetate	214.5	Sigma	M-2545	RT
MgCl <sub>2</sub>	203.3	Sigma	M-2670	RT, dessicator
Oligomycin	750.62	Sigma	O-4876	-20 C / Dessicator
Palmitoyl-L-carnitine	436.1	Sigma	P-1645	-20 C / Dessicator
Pyruvate	110	Sigma	P-2256	+ 4 C/ Dessicator
Saponin		Sigma	S-4521	RT
Taurine	125.15	Sigma	T-0625	RT
Sodium succinate	270.1	Sigma	S-5047	RT
DMSO	78.13	Sigma	D-2650	RT
Rotenone	394.4	Sigma	R-8875	RT (cover with Al foil)
Sodium Hydrosulfite		Sigma	157953	RT (cover aliquot with Al foil)