MitoCheck® Mitochondrial OCR Assay Kit

Item No. 701170

Customer Service 800.364.9897 * **Technical Support** 888.526.5351
www.caymanchem.com

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GENERAL INFORMATION

Materials Supplied

Kit will arrive packaged at -20°C. Remove components and store as indicated.

Item No.	Item	Quantity/Size	Storage
701171	Mitochondrial Respiration Buffer	40 ml	-20°C
701172	MitoCheck® OCR Succinate Assay Reagent	1 ml	-20°C
701173	MitoCheck® ADP Assay Reagent	200 µl	-20°C
701174	Oligomycin Assay Reagent	100 µl	-20°C
600801	MitoXpress®-Xtra	1 ea	4°C
701175	Light Mineral Oil	15 ml	RT
701176	MitoCheck® Antimycin A Assay Reagent	100 µl	-20°C
700933	Fatty Acid Free-BSA Assay Reagent	250 µl	-20°C

NOTE: MitoXpress® - Xtra is a product of Luxcel Biosciences.

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

NOTE: It is recommended that gloves be worn at all time when working with isolated mitochondria and mitochondrial inhibitors.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Fax: 734-971-3641

Email: techserv@caymanchem.com

Hours: M-F 8:00 AM to 5:30 PM EST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

The MitoXpress® - Xtra vial may be stored in the following manner:

Dry material: store between +2 to +8°C (until the indicated expiration date). **Reconstituted product:** can be stored aliquoted at -20°C. Avoid freeze/thaw cycles and use within one month. Protect products from prolonged exposure to light.

This kit will perform as specified if stored as directed in the **Materials Supplied** section and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. Clean glassware; washed with 70% ethanol in place of soap (to ensure the removal of detergent).*
2. A refrigerated centrifuge and rotor capable of 1,000 x g and 10,000 x g.*
3. A pH meter, and solutions of HCl (1 M) and KOH (1 M).*
4. Surgical scissors*
5. A polytron or tissumiser (for heart and muscle tissue).*
6. SpectraMesh nylon mesh filter (300 µm).*
7. A 50 ml glass Dounce homogenizer with loose pestle (A) and a 2 ml glass or Teflon homogenizer with loose pestle.*
8. A glass rod*
9. Polycarbonate centrifuge tubes (4x30 ml) capable of withstanding 10,000 x g.*
10. Protein assay (Lowry or BCA).*
11. A 1 mM stock of rotenone prepared in pure non-denatured ethanol.
12. A plate reader capable of measuring fluorescence using excitation and emission wavelengths of 380 and 650 nm, respectively, and having plate temperature control.
13. Adjustable pipets and/or multichannel pipets.
14. A repeater pipept
15. 96-well (black) clear bottom tissue culture plates or standard clear polystyrene plates.

* Required for MitoCheck (Tissue) Isolation Kit (Item No. 701010) (Not supplied)

NOTE: This kit was designed and optimized to work with the MitoCheck Mitochondrial (Tissue) Isolation Kit (Item No. 701010) (Not Included). Users wishing to use their own mitochondrial isolation procedure with this kit may do so at their discretion. For convenience, the protocol for the MitoCheck Mitochondrial (Tissue) Isolation Kit is provided within this kit booklet.

INTRODUCTION

About This Assay

Cayman's Mitocheck® Mitochondrial OCR Assay Kit is designed to measure OCR directly in freshly isolated mitochondria. This kit combines a mitochondrial respiration buffer, substrates, and inhibitors with the phosphorescent oxygen probe MitoXpress®-Xtra to accurately determine mitochondrial OCR in a high throughput manner. While there are several well-established methods that allow for the measurement of cellular OCR in cells, these methods only accurately reflect the respiration rates of the cell and may not necessarily represent specific mitochondrial function. For convenience, a mitochondrial tissue isolation procedure is outlined in this kit. However, the buffers needed to perform the isolation, which are available in Cayman's Mitocheck® (Tissue) Mitochondrial Isolation Kit (Item No. 701010), must be purchased separately.

Properties of MitoXpress® - Xtra

MitoXpress® - Xtra is a chemically stable and inert biopolymer-based cell-impermeable probe. Excitation and emission information can be found in Figure 1. MitoXpress®-Xtra phosphorescent lifetime signal increases as the oxygen concentration decreases. These properties make the probe ideal for time resolved fluorescence measurements which can offer an increased signal under conditions where background is high.

	Peak Maxima (nm)	Peak (nm)
Excitation*	380	360-400
Emission	650	630-680

*Excitation at 532 ±7.5 nm is also possible.

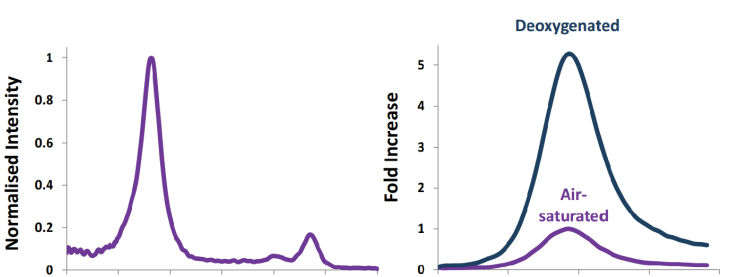


Figure 1. Excitation and Emission spectrums of MitoXpress®-Xtra
Left panel: shows normalized excitation spectrum of MitoXpress® - Xtra, with emission at 650 nm. Excitation maxima are observed at 380 or 532 nm. *Right panel:* shows emission spectrum of MitoXpress® - Xtra in oxygenated (purple line) and deoxygenated (black line) conditions with excitation at 380 nm. Under the conditions of measurement, signal increased 5-fold on deoxygenation.

Fluorescence Measurements

There are three available options for measuring fluorescence:

1. Standard fluorescence intensity measurement
2. Time-resolved fluorescence (TR-F) measurement
3. Ratiometric TR-F measurement (subsequent Lifetime calculation)

The MitoXpress® - Xtra probe can be measured with standard fluorescence intensity or TR-F measurements, using monochromator or filter based plate-readers. TR-F measurement reduces non-specific background and increases probe sensitivity, offering a more stable reading and wider dynamic range than measuring fluorescence intensity. Ratiometric TR-F measurement can be used to maximize dynamic range and assay performance. To determine which measurement mode is best suited for your instrument please refer to Table 1 on page 12, visit luxcel.com/Instrument+Measurement+Parameters, or consult your instrument manufacturer.

1. **Standard Measurement**

   Optimal wavelengths are 380 nm for excitation and 650 nm for emission. Please refer to Table 1 on page 12 for instrument specific settings. *NOTE: This option can often result in a lower signal to background. Time resolved measurements may be performed to improve signal to background.*

2. **TR-F Measurement**

   Optimal wavelengths are 380 nm for excitation and 650 nm for emission with a recommended delay time of 30 µs. Please refer to Table 1 on page 12 for instrument specific settings.

3. Ratiometric TR-F (Lifetime) Measurement

Ratiometric TR-F allows for the calculation of lifetime using dual time resolved measurements. In this mode, two separate time resolved readings (W_1 and W_2) are taken. From these values, a lifetime is calculated using the equation below. *NOTE: For accurate calculation of lifetime, ensure that gain values for W_1 and W_2 are identical. Please refer to table 1 on page 12 for instrument specific settings.*

Lifetime Calculation: Use the dual intensity readings and the following transformation to calculate the corresponding Lifetime (µs):

$$\text{Lifetime (µs) } [\tau] = (70\text{-}30)/\ln(W_1/W_2)$$

Where W_1 and W_2 represent window 1 and 2, respectively, for the measured intensity readings at each time point, and 70 and 30 represent the delay time of W_2 and W_1, respectively. This provides Lifetime values in µs at each measurement.

Example calculation:

W_1 = 75,629 counts and W_2 = 14,654 counts

Lifetime = (70-30)/ln(75,629/14,654)

Lifetime = 24.4 µs

Lifetime Signal should be in the range ~22 to ~68 µs. Lifetime values should only be calculated from samples containing MitoXpress® - Xtra probe. Lifetime values should not be calculated from blank wells.

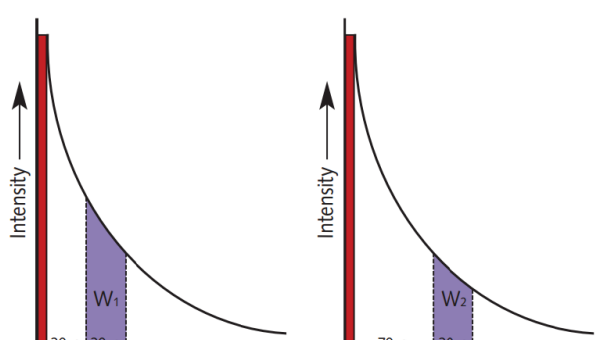


Figure 2. Illustration of ratiometric TR-F measurement

	FLUOStar & POLARstar Omega (BMG Labtech)	Victor series X3, X4, X5 (Perkin Elmer)	FLUOStar & POLARstar Optima (BMG Labtech)	Infinite/Safire/ Genios Pro (Tecan)	SpectraMax/ Flexstation/ Gemini (Mol. Devices)
Light source	Xe-flashlamp	Xe-flashlamp	Xe-flashlamp	Xe-flashlamp	Xe-flashlamp
Optical Configuration	Filter-based Top/Bottom reading	Filter-based Top reading	Filter-based Top/Bottom reading	Filter-based Top/ Bottom reading	Monochromator-based Top/ Bottom reading
Measurement mode	*Ratiometric TR-F	*Ratiometric TR-F	TR-F	TR-F	Standard
Excitation	380 ±20 nm (TR-EX L)	340 ±40 nm (D340)	380 ±20 nm (TR-EX L)	380 ±20nm	380 nm
Emission	650 ±50 nm (BP-650)	642 ±10 nm (D642)	650 ±50 nm (BP-650)	650 ±20 nm	650 nm
Delay time 1	30 µs	30 µs	30 µs	30 µs	N/A
**Delay time 2	70 µs	70 µs	N/A	N/A	N/A
Read time 1	30 µs	30 µs	100 µs	100 µs	N/A
Read time 2	30 µs	30 µs	N/A	N/A	N/A

Table 1. Recommended Instrument and Measurement settings.*
**NOTE: A more comprehensive and current version of this table can be found at: www.luxcel.com/Instrument+Measurement+Parameters.*
TR-F, time-resolved fluorescence
*TR-F attachment installed in instrument
**Applicable to ratiometric TR-F measurement only.

NOTE: Preset Protocol Files for BMG instruments are available from www.luxcel.com and BMG Technical Support.

PRE-ISOLATION PREPARATION

Reagent Preparation

Isolation Protocol - For MitoCheck Mitochondrial (Tissue) Isolation Kit - Item No. 701010 (not supplied)

1. **Mitochondrial Isolation Buffer**

   This buffer is supplied as a 5X concentrate. Before using, dilute to 1X by adding 200 ml of cold, ultrapure water. Check pH at 4°C and ensure that it is at 7.4 before beginning. If pH is slightly high, adjust with concentrated HCl. If buffer becomes too acidic, adjust using KOH. Do not use NaOH. This component will make 250 ml of 1X Mitochondrial Isolation Buffer.

2. **Mitochondrial Homogenization Buffer**

   This buffer is supplied as a 5X concentrate. Before using, dilute to 1X by adding 80 ml of cold, ultrapure water. Check pH at 4°C and ensure that it is at 7.4 before beginning. If pH is slightly high, adjust with concentrated HCl. If buffer becomes too acidic, adjust using KOH. Do not use NaOH. This component will make 100 mL of 1X Mitochondrial Homogenization Buffer.

Oxygen Consumption Protocol

1. **Mitochondrial Respiration Buffer - (Item No. 701171)**

   This buffer is ready to use as supplied, however some precipitation upon thawing is normal. To dissolve precipitate, warm to 37°C and vortex vigorously until particles have dissolved. Store any unused reagent at -20°C.

2. **MitoXpress®Xtra - (Item No. 600801)**

   Reconstitute lyophilized powder in 1 ml of Mitochondrial Respiration Buffer. Triturate multiple times to ensure MitoXpress®-Xtra is fully dissolved. The reconstituted MitoXpress® - Xtra solution is stable for one day when stored at 4°C. For long term storage, aliquot the reconstituted solution and store at -20°C, which is stable for one month.

3. **MitoCheck® OCR Succinate Assay Reagent - (Item No. 701172)**

   This vial contains a 0.5 M solution of succinate. To prepare an 80 mM working solution of succinate, dilute 640 µl of Succinate Assay Reagent in 3360 µl of Mitochondrial Respiration Buffer. Keep on ice until use. Store any unused reagent at -20°C.

4. **MitoCheck® ADP Assay Reqagent - (Item No. 701173)**

   This vial contains a 0.25 M solution of ADP. Once thawed, this vial is ready to use as supplied. Vortex thoroughly before use. Keep on ice until use. Store any unused reagent at -20°C.

5. **Oligomycin Assay Reagent - (Item No. 701174)**

   This vial contains a 1 mg/ml solution of oligomycin in non-denatured ethanol. Wear appropriate personal protective equipment when handling mitochondrial inhibitors. To prepare a 8 µg/ml working solution of Oligomycin, dilute 32 µl of the Oligomycin Assay Reagent into 3,968 µl of Mitochondrial Respiration Buffer. Keep on ice until use. Store any unused reagent at -20°C.

6. **MitoCheck® Antimycin A Assay Reagent - (Item No. 701176)**

   This vial contains a 10 mM solution of Antimycin A in non-denatured ethanol. Wear appropriate personal protective equipment when handling mitochondrial inhibitors.To prepare 80 µM working solution of Antimycin A, dilute 32 µl of the Antimycin A Assay Reagent into 3,968 µl of Mitochondrial Respiration Buffer. Keep on ice until use. Store any unused reagent at -20°C.

7. **State 3 Respiration Buffer* prepare post-isolation**

   The following table can be used to prepare State 3 Respiration Buffer, which contains ADP. In coupled mitochondria, the addition of ADP increases respiration rate as the mitochondria phosphorylate ADP to form ATP. It is converse to state 2 or 4 respiration rates, where the respiration rates of quiescent (non-phosphorylating) mitochondria are measured. This protocol will yield enough State 3 Respiration Buffer for 136 wells. State 3 Respiration Buffer is stable on ice for two hours in the absence of mitochondrial protein. Once mitochondrial protein has been added, State 3 Respiration Buffer should be kept on ice, and used within 30 minutes. Any unused State 3 Respiration Buffer should be discarded.

Reagent	Volume	Final Concentration (in well)
Mitochondrial Respiration Buffer	To 15 mL	-
ADP Assay Reagent (Omit for state 2 respiration)	175 µl	2 mM
Fatty Acid Free-BSA Assay Reagent	22 µl	0.05%
Rotenone (not supplied)*	22 µl	1 µM

Table 2. Pipetting summary for the preparation of State 3 Respiration Buffer. *The use of rotenone is optional, but recommend as omission of rotenone may result in damage to mitochondria from increased ROS due to backflow of electrons *via* complex I. Additionally, electron backflow may also result in reduced oxygen consumption rates.

ASSAY PROTOCOL

Isolation Protocol

NOTE: This protocol is for use only with the MitoCheck Mitochondrial (Tissue) Isolation Kit (Item No. 701010) and should not be used with other mitochondrial preparations. Ensure all glassware, centrifuge tubes, centrifuge and rotor are pre-chilled prior to use. All steps are carried out at 4°C.

1. Starting with 1-20 g wet weight of freshly isolated tissue, place into a cold 100 ml beaker with ~10 ml of Mitochondrial Isolation Buffer. *NOTE: This protocol is designed for use with varying amounts of starting material. Mitochondrial protein yield will vary based on mitochondrial content and overall mass of starting material.*

Tissue Specific Preparations:

For Liver: chop finely. For Kidney: remove renal capsules and chop finely, allow tissue to settle, decant blood and rinse with ~20 ml of Mitochondrial Isolation Buffer. A small amount of tissue loss during this step is expected.

i. Repeat wash/decant step 2 more times, until blood is removed.

ii. Suspend tissue in Mitochondrial Homogenization Buffer and transfer to pre-chilled 50 ml Dounce homogenizer.

iii. Fill to just above the neck (~30 ml) with Mitochondrial Homogenization Buffer and homogenize using the loose fitting pestle (Pestle A) (on ice) for 10 passes (1 pass - 1 down stroke, 1 up stroke).

iv. *Quickly* transfer the homogenate evenly into two 30 ml polycarbonate centrifuge tubes and fill with Mitochondrial Homogenization Buffer.

v. Centrifuge for 3 minutes at 1,000 x g (RCF).

vi. Taking care not to dislodge the pellet, leaving a small amount behind, carefully decant the supernatant into two clean 30 ml polycarbonate centrifuge tubes and fill with Mitochondrial Homogenization Buffer. It may be helpful to leave behind a few milliliters of supernatant so that excess blood is not transferred over, then proceed to step 2.

For Heart and Muscle. *NOTE: Lower mitochondrial yields are expected with these tissues due to intense homogenization*. Chop finely and allow tissue to settle, decant blood, and rinse with ~10 ml of Mitochondrial Isolation Buffer. A small amount of tissue loss during this step is expected.

i. Suspend tissue in 10 ml of Mitochondrial Homogenization Buffer and homogenize with tissuemizer for 20 seconds. Transfer homogenate to a 30 ml centrifuge tube.

ii. Centrifuge for five minutes at 600 x g (RCF).

iii. Transfer the supernatant through 300 µm mesh into a clean 30 ml centrifuge tube.

iv. Homogenize pellet with tissuemizer as in step i.

v. Repeat steps iii and iv.

vi. Fill each centrifuge tube with Mitochondrial Homogenization Buffer and proceed to step 2.

2. Centrifuge for 10 minutes at 10,000 x g (RCF).
3. Discard the supernatant (should appear cloudy) and add ~ 20 ml of Mitochondrial Isolation Buffer to the centrifuge tube. Use the glass rod to suspend the mitochondrial pellet and then fill tube with Mitochondrial Isolation Buffer.
4. Repeat Step 2.
5. Supernatant should be now be clearer than that of the step 1. Add 10 ml of Mitochondrial Isolation Buffer to each centrifuge tube and suspend with the glass rod. Combine the suspension into one centrifuge tube. Fill with Mitochondrial Isolation Buffer and repeat steps 2, 3 and 4.
6. Following completion of the 10 minute, 10,000 x g (RCF) centrifugation step, discard the supernatant and suspend the pellet in ~300 µl of Mitochondrial Isolation Buffer. Transfer the suspension to a pre-chilled 2 ml Dounce homogenizer. Homogenize three strokes with the loose fitting pestle. Remove an aliquot for protein determination.
7. Freshly isolated mitochondria should be stored on ice in a 4 ml glass test tube. For coupled mitochondrial studies oxygen consumption studies, use within four hours of isolation.

NOTE: Freshly isolated mitochondria cannot be frozen and still maintain their coupled state.

Oxygen Consumption Protocol

Typical Instrument Set Up

NOTE: Instrument settings will vary between manufacturers. Please refer to http://luxcel.com/Instrument+Measurement+Parameters for a list of optimal settings for common plate readers.

1. Set the plate reader temperature control to 37°C.
2. Optimal wavelengths are 380 ±20 nm for excitation and 650 ±20 nm for emission.
3. For TR-F or ratiometric TR-F, delay and mesurment times refer to Table 1 on page 12 or *www.luxcel.com/Instrument+Measurement+Parameters* for the parameters best suited for your plate reader.
4. Gain should be optimized so that the fluorescent signal of MitoXpress® in 21% O_2 (air saturated) buffer is equal to 20% of the maximum detectable signal.

Instrument Signal Optimization

To optimize the signal, the following steps should be performed. For standard measurements or TR-F measurements, a signal to blank ratio ≥3 is required. For ratiometric TR-F (lifetime) measurement, a signal to blank ratio ≥10 is required for W2 (see Ratiometric TR-F (Lifetime) Measurement on page 10).

1. In a spare black, clear bottom 96-well tissue culture treated plate, add 150 µl of Respiration Buffer to six wells.
2. Add 10 µl of Mitochondrial Respiration Buffer to three of these wells. These are your blank signal wells.
3. Add 10 µl of MitoXpress® - Xtra Solution (Page 13) to the other three wells. These are your signal wells.
4. Gently overlay each well with 100 µl of Light Mineral Oil (Item No. 701175). The use of a repeating pipette is preferred.
5. Read the plate immediately with the set up described on page 18. The plate should be measured kinetically for 30 minutes to ensure the fluorescent signal is stable.
6. If required, adjust the instrument parameters to increase measurement sensitivity in order to achieve maximal S/B ratio. The following options may be helpful:

   -Increase gain (or PMT) settings or flash energy

   -Adjust TR-F focal height

   -Repeat as a top, or bottom read (plate reader dependent)

   -Increase volume of MitoXpress® - Xtra from 10 µl to 15 µl

   -Contact Instrument supplier for further options

Performing the Assay

Using State 3 Respiration Buffer, dilute mitochondria so that the in-well mitochondrial protein concentration does not exceed 1 mg/ml. We recommend testing range of protein concentrations to avoid rapid consumption of O_2 by mitochondrial samples. Save 400 µl of State 3 Respiration Buffer without mitochondria to be used in Background Signal Wells.

Reagent	Volume (µl)
State 3 Respiration Buffer	110
MitoXpress®-Xtra	10
Test compounds (Oligomycin, Antimycin A, or Mitochondrial Respiration Buffer or Unknowns)	20
Succinate (add last to all wells to initialize)	20
Total Volume	160

Table 3. Pipetting summary for each well.*

1. To all wells except for the Background Signal Wells, add 110 µl of State 3 Respiration Buffer containing diluted mitochondrial samples.
2. For Background Signal Wells, add 110 µl State 3 Respiration Buffer without mitochondria. Add 10 µl of MitoXpress®-Xtra to all wells.
3. Add 20 µl of the compound to be tested (See Table 3):
   - To measure state 3 respiration rate, add Mitochondrial Respiration Buffer.
   - To measure state 4 respiration, add diluted Oligomycin.
   - For full inhibition, add diluted Antimycin A.
   - To test for mitochondrial inhibition of an unknown chemical, add the unknown diluted in Mitochondrial Respiration Buffer.
     Note: Solvent concentration should not exceed 1%.
4. After adding all test compounds, add 20 µl succinate to all wells to initialize the assay. It is recommended that succinate be added using a multichannel pipette.
5. After the addition of succinate, overlay all wells with 100 µl of pre-warmed mineral oil. This can be easily accomplished with a repeater pipette.

*It is recommended that all pipetting take place on a heat block set to 37°C. This will ensure temperature is kept constant. It is also recommended that all conditions be performed in triplicate.

ANALYSIS

Calculations

Assessing Oxygen Consumption

Plot the MitoXpress®-Xtra Signal, Intensity, or Lifetime *versus* Time (mins) (see Figure 3 on page 23). Select the linear portion of the signal profiles and apply linear regression to determine the slope for each of the signal profiles. (This approach is preferable to calculating a slope from averaged profiles.)

Tabulate the slope values for each sample and calculate appropriate average and standard deviation values. The slope obtained for the **Background** Wells (sample without mitochondria) should be subtracted from all test values.

Performance Characteristics

Sample Data

The data shown below are an example of data obtained with this kit. Your results will not be identical to these. Do not use these data to directly compare your samples as your results may vary substantially.

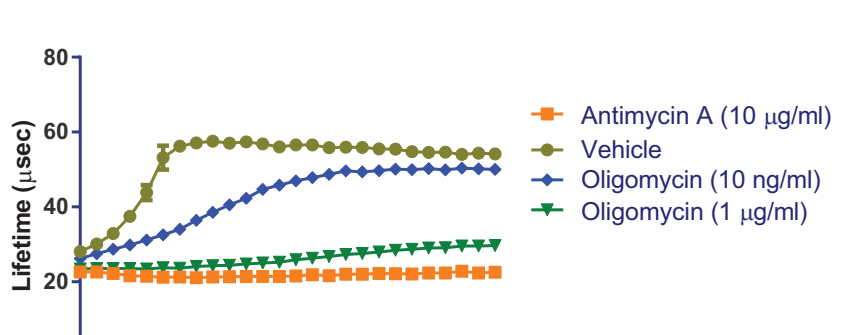


Figure 3. Sample Data
Oxygen consumption is measured in isolated mouse liver mitochondria (0.12 mg/ml) in the presence the complex V inhibitor oligomycin (1 µg/ml, 10 ng/ml), the complex III inhibitor antimycin A (10 µM) and vehicle control. The difference in oxygen consumption rates in the absence and presence of oligomycin indicate that these mitochondria are coupled.

Troubleshooting

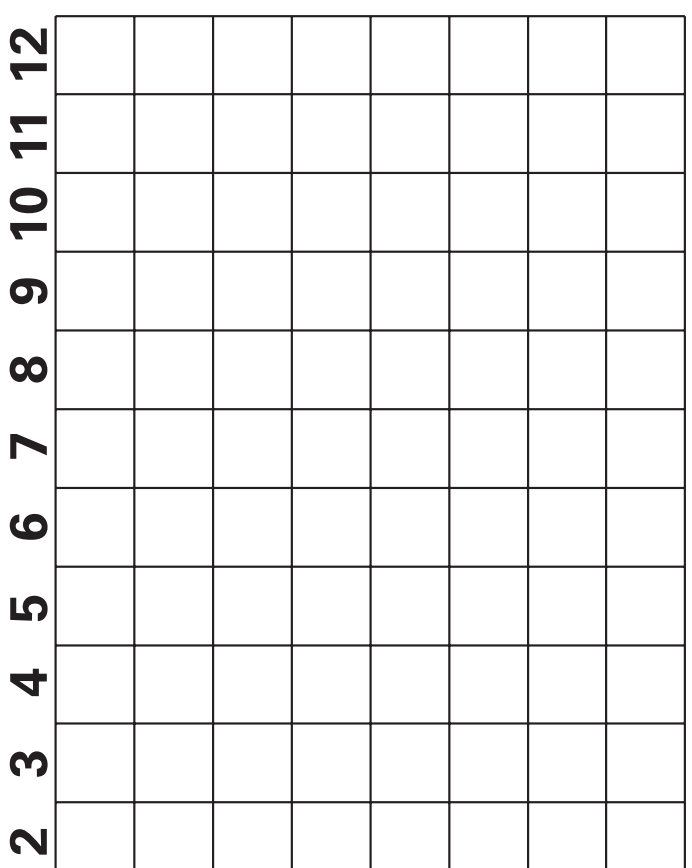
Problem	Possible Causes	Recommended Solutions
Poor mitochondrial protein yield	Tissue not thoroughly homogenized	Ensure that no solid pieces of tissue are visible post homogenization as this can lead to decrease yield
Poor RCR or uncoupled mitochondria	A. Tissue homogenized too thoroughly B. Detergent in glassware C. Method of euthanasia	A. Be sure to use loose pestle and homogenize to the point where no tissue pieces are visible. Excess homogenization/ use of tight pestle can result in the shearing of mitochondria B. Ensure that all glassware that that comes into contact with buffers has been washed with ethanol and ultrapure water C. Excess stress both prior to and during euthanasia can result in mitochondrial uncoupling due to the release of adrenaline
Excess blood in mitochondrial pellets	A. Tissue not washed thoroughly prior to homogenization B. Some of the pellet from the low speed spin has been carried over	A. Ensure that all excess blood has been removed by washing tissue pieces with homogenization buffer until surrounding buffer is clear B. Leave a small amount of supernatant behind to ensure that pellet is not disturbed

Troubleshooting cont.

Problem	Possible Causes	Recommended Solutions
Sudden drop in fluorescent signal when starting assay	Temperature variation in plate	Reduce plate preparation time to <10 minutes; use plate heater during plate preparation
No oxygen consumption observed	Low mitochondrial protein concentration	Increase mitochondrial content

References

1. Smith, A.L. Preparation, properties, and conditions for assay of mitochondria: Slaughterhouse material, small-scale. *Method. Enzymol.* **10**, 81-86 (1967).
2 Fleischer, S., Rouser, G., Fleischer, B., *et al.* . *J. Lipid Res.* **8(3)**, 170-180 (1967).



NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.