Oxygen Consumption Rate Assay Kit (MitoXpress® - Xtra HS Method)

Item No. 600800

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

TABLE OF CONTENTS

GENERAL INFORMATION

Materials Supplied

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

Item Number	Item	Quantity/Size	Storage
600801	MitoXpress® - Xtra	1 vial	4°C in the dark
660910	HS Mineral Oil Assay Reagent	1 vial/15 ml	Room Temperature in the dark
600802	Cell-Based Assay Glucose Oxidase	1 vial/2 mg	-20°C
600803	Cell-Based Assay Antimycin A	1 vial/200 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 laboratory research use only: not for administration to humans. Not for human or veterinary diagnostic or therapeutic use.

Precautions

Please read these instructions carefully before beginning this assay.

For research use only. Not for human or diagnostic use.

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. A plate reader capable of measuring fluorescence using excitation and emission wavelengths of 380 and 650 nm, respectively, and having plate temperature control.
2. Adjustable pipettes and a repeating pipette.
3. 96-well (black) clear bottom tissue culture plates or standard clear polystyrene plates for culturing cells.

INTRODUCTION

Background

Cellular homeostasis is maintained through the generation of ATP. The generation of ATP can be accomplished through glycolysis alone (anaerobic respiration) or through the coupling of glycolysis to oxidative phosphorylation. Oxidative phosphorylation, which is oxygen (O_2) dependent, takes place in the mitochondrion and is the most efficient and preferred means of ATP synthesis by mammalian cells. Thus, the oxygen consumption rate (OCR) of cells is an important indicator of normal cellular function. Unhealthy cells with dysfunctional mitochondria show a lower oxygen consumption rate compared to healthy cells.

Measurement of oxygen consumption has classically been achieved through the use of a Clark-type oxygen electrode. However, this method has limitations, as it requires specialized equipment and has a low sample throughput. Recently, a phosphorescent oxygen probe, MitoXpress® - Xtra, developed by Luxcel Biosciences, has proven to be useful in measuring oxygen consumption rates in whole cells.[1] The phosphorescent signal of MitoXpress® - Xtra is quenched by oxygen and resulting in a signal that is inversely proportional to the amount of oxygen present. Additionally, the signal lifetime is also quenched by molecular O_2. The probe can be used in a time resolved mode, minimizing background florescence. Using the data obtained, the cellular OCR can then be calculated from the changes in the MitoXpress® - Xtra probe signal over time.[2]

About This Assay

Cayman's cell-based Oxygen Consumption Rate Assay Kit (MitoXpress® - Xtra HS Method) utilizes this newly developed phosphorescent oxygen probe to measure OCRs in living cells. The MitoXpress® - Xtra probe functions best in samples containing oxygen concentrations from 0-21%. Antimycin A, an inhibitor of the mitochondrial electron transport chain, is included and is to be used as a control for zero oxygen consumption (low signal). Glucose oxidase is also included in the kit to be used as a reference for oxygen depletion (high signal).

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.

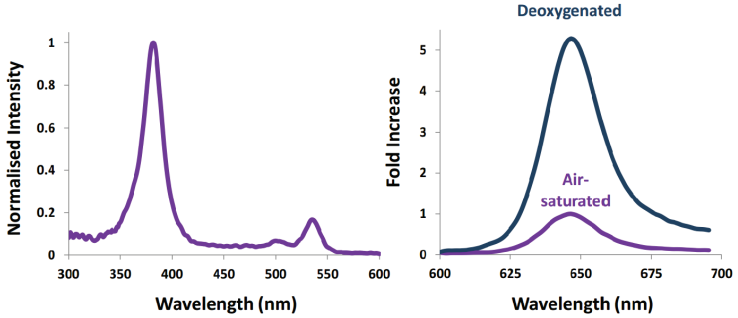


Figure 1. Excitation and Emission spectrums of MitoXpress® - Xtra
Left panel: shows normalized excitation spectrum of MitoXpress® - Xtra, with emission at 650 nm. Excitation maxima are observed at 380 or 532 nm. *Right panel:* shows emission spectrum of MitoXpress® - Xtra in oxygenated (purple line) 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 10, 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 10 for instrument specific settings. *NOTE: This option can often result in a lower signal to background. Time resolved measurements 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 10 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 10 for instrument specific settings.*

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

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

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.

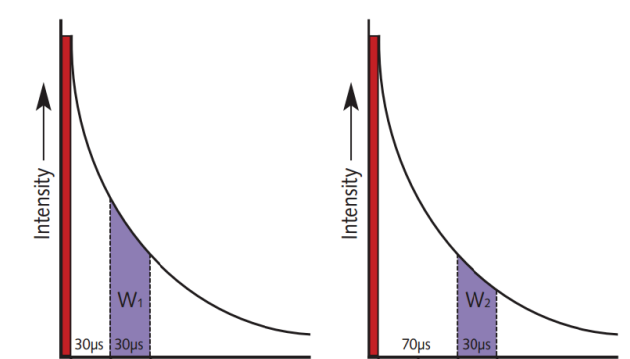


Figure 2. Illustration of ratiometric TR-F measurement

	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.
A more comprehensive 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-ASSAY PREPARATION

Reagent Preparation

1. MitoXpress® - Xtra Solution

Prior to use, reconstitute the contents of the MitoXpress® - Xtra vial (Item No. 600801) with 1 ml of distilled water or assay medium. 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. The MitoXpress® - Xtra will be stable for one month when stored at -20°C.

2. Glucose Oxidase Stock Solution

Prior to use, reconstitute the contents of the Cell-Based Assay Glucose Oxidase vial (Item No. 600802) with 0.2 ml of distilled water. For long term storage, aliquot the reconstituted solution and store at -20°C. The reconstituted stock solution will be stable for two months when stored at -20°C.

3. Antimycin A Stock Solution

Prior to use, thaw the Cell-Based Assay Antimycin A vial (Item No. 600803) and warm to room temperature. The Antimycin A will be stable for at least one year if stored at -20°C. *NOTE: Please ensure that proper personal protective equipment is worn when handling Antimycin A.*

ASSAY 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 10 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 8).

1. In a spare black, clear bottom 96-well tissue culture treated plate, add 140 µl of culture medium to six wells.
2. Add 10 µl of culture medium to three wells. These are your blank signal wells.
3. Add 10 µl of MitoXpress® - Xtra Solution (Page 11) to three wells. These are your signal wells.
4. Gently overlay each well with 100 µl of HS Mineral Oil (Item No. 660910). The use of a repeating pipette is preferred.
5. Read the plate immediately with the set up described on page 12. 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 without phenol red or serum

   -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

Plate Set Up

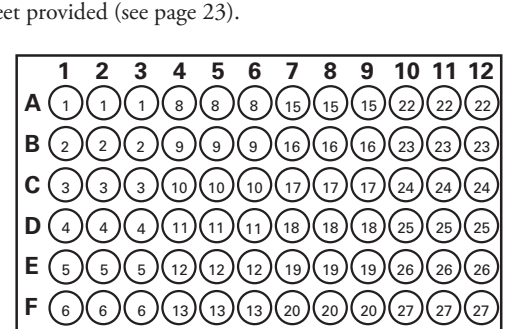
There is no specific pattern for using the wells on the plate, but it is important to include the following control wells containing no cells:

Blk - Background Wells containing culture medium overlaid with oil.

MX - MitoXpress® Signal Wells containing culture medium plus MitoXpress® - Xtra Solution overlaid with oil.

GO - Glucose Oxidase Wells containing culture medium, Glucose Oxidase Solution, and MitoXpress® - Xtra Solution overlaid with oil.

Antimycin A (**AA**) wells contain cells treated with Antimycin A Stock Solution. Sample wells contain cells treated with experimental compounds or vehicle. We recommend that each treatment be performed in triplicate and that you record the contents of each well on the template sheet provided (see page 23).



1-28 = Sample Wells
Blk = Blank/Background Wells, containing no cells
MX = MitoXpress® - Xtra Wells, containing no cells
GO = Glucose Oxidase Wells, containing no cells
AA = Antimycin A Wells

Figure 3. Sample plate format

Performing the Assay

Pipetting Hints

- It is recommended that a multichannel pipette be used to deliver reagents to the wells. This saves time and helps maintain more precise incubation times.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

1. Seed cells in a black, clear bottom 96-well tissue culture treated plate at a density of 40,000-80,000 cells/well in 200 µl of culture medium. *NOTE: Optimal seeding density will vary based on aerobic capacity of the cell line. We recommend trying a range of cell densities to optimize oxygen consumption rates.* Incubate the cells overnight using appropriate culture conditions for the experimental cell type. It is important to have nine wells with no cells for the controls described in **Plate Set Up**.
2. Remove spent culture medium from all wells and replace with 150 µl of fresh medium.
3. Add test compounds or the appropriate vehicle in 10 µl to Sample Wells. *NOTE: To assess the effect of a compound on mitochondrial function, cells are typically treated immediately prior to measurement. Prolonged incubations with test compounds can be performed if required.* After prolonged treatment remove spent culture medium from all wells and replace with 150 µl of fresh medium.
4. Add 20 µl of culture medium to the three Blk Wells.
5. Add 10 µl of Glucose Oxidase Stock Solution (Page 11) to the **GO** Wells.
6. Add 10 µl of Antimycin A Stock Solution (Page 11) to the **AA** Wells.
7. Add 10 µl of culture medium to the **MX** Wells.
8. Add 10 µl of MitoXpress® - Xtra Solution (Page 11) to every well ***except*** the three **Blk** Wells.
9. Gently overlay every well with 100 µl of HS Mineral Oil (Item No. 660910). The use of a repeating pipette is preferred. *NOTE: Ensure the HS Mineral Oil is pre-warmed to the measurement temperature.*
10. Read the plate immediately with the set up described on page 12. The plate should be measured kinetically for ≥120 minutes.

Wells	Culture Medium (µl)	Glucose Oxidase (µl)	Antimycin A (µl)	Test Compounds (µl)	Extra Culture Medium (µl)	MitoXpress® - Xtra (µl)
Sample	150	-	-	10		10
Blk	150	-	-		20	-
MX	150	-	-		10	10
GO	150	10	-		-	10
AA	150	-	10		-	10

Table 2. Pipetting summary

ANALYSIS

Calculations

Assessing Oxygen Consumption

Plot the MitoXpress® - Xtra Signal, Intensity, or Lifetime *versus* Time (mins) (see Figure 4 on page 18). 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 **Blk** Wells (sample without cells) should be subtracted from all test values.

Plotting the Dose Response Curve

To generate dose response data, plot the data generated as outlined above against the corresponding compound concentration, see Figure 5 on page 19.

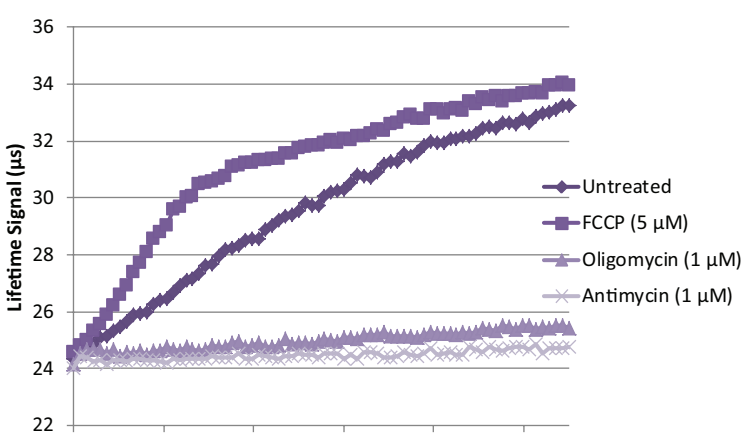


Figure 4. Typical Lifetime signal profile of MitoXpress® - Xtra for cell samples which have been treated with different classical electron transport chain inhibitor or activator compounds.

Performance Characteristics

The dose response curve presented here is an example of the data typically produced with the assay.

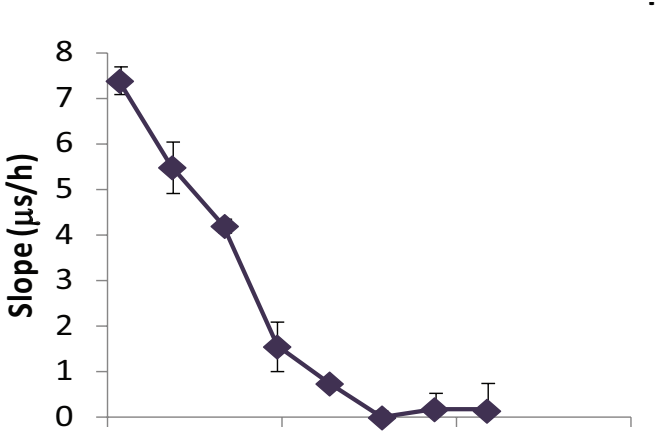


Figure 5. Dose response curve
Antimycin A concentration (µM) *versus* calculated slope (µs/hour), showing Antimycin A causes an inhibitory response on cellular oxygen consumption.

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Signals indistinguishable from blanks	Incompatible instrument or incorrect instrument settings	Check instrument suitability and setup and run proper controls without cells (S/B test) (probe/no probe)
Signals detectable, but signal changes too small	Instrument performance is poor (low S/B ratio); monolayer cell density used is too low	Check the instrument and run proper controls; use greater cell density; optimise assay conditions
There is a drop in signal over the initial minutes	Plate temperature equilibration; baseline drift	Use plate block heater during plate preparation; pre-warm all solutions
Initial intensity is inconsistent	Long plate preparation times	Reduce plate preparation time to <10 minutes; use plate heater during plate preparation

Reference

1. Hynes, J., Marroquin, L.D., Ogurtsov, V.I., *et al.* Investigation of drug-induced mitochondrial toxicity using fluorescence-based oxygen-sensitive probes. *Toxicol. Sci.* **92(1)**, 186-200 (2006).
2 Hynes, J., Natoli, E., Jr., and Will, Y. Fluorescent pH and oxygen probes of the assessment of mitochondrial toxicity in isolated mitochondria and whole cells. *Curr. Protoc. Toxicol.* 2.16.1-2.16.22 (2009).

Related Products

Annexin V FITC Assay Kit - Item No. 600300
Caspase-3 Fluorescence Assay Kit - Item No. 10009135
Caspase-3 (human) Polyclonal Antibody - Item No. 160745
Caspase-9 Polyclonal Antibody - Item No. 160790
Glucose Uptake Cell-Based Assay Kit - Item No. 600470
Glycolysis Cell-Based Assay Kit - Item No. 600450
Intracellular O_2 Respiratory Burst Imaging Kit - Item No. 601020
JC-1 Mitochondrial Membrane Potential Assay Kit - Item No. 10009172
LDH Cytotoxicity Assay Kit - Item No. 601170
MitoCheck Citrate Synthase Activity Assay Kit - Item No. 701040
MitoCheck Complex I Activity Assay Kit - Item No. 700930
MitoCheck Complex II Activity Assay Kit - Item No. 700940
MitoCheck Complex II/III Activity Assay Kit - Item No. 700950
MitoCheck Complex IV Activity Assay Kit - Item No. 700990
MitoCheck Complex V Activity Assay Kit - Item No. 701000
MitoCheck Mitochondrial (Tissue) Isolation Kit - Item No. 701010
MTT Cell Proliferation Assay Kit - Item No. 10009365
Multi-Parameter Apoptosis Assay Kit - Item No. 600330
NAD/NADH Cell-Based Assay Kit - Item No. 600480
Nitric Oxide Cell-Based Assay Kit - Item No. 10009419
Oxygen Consumption/Glycolysis Dual Assay Kit - Item No. 601060
Oxygen Consumption/MitoMembrane Potential Dual Assay Kit - Item No. 600880
PURO-Fluor Protein Synthesis Labeling Kit - Item No. 601100
WST-1 Cell Proliferation Assay Kit - Item No. 10008883

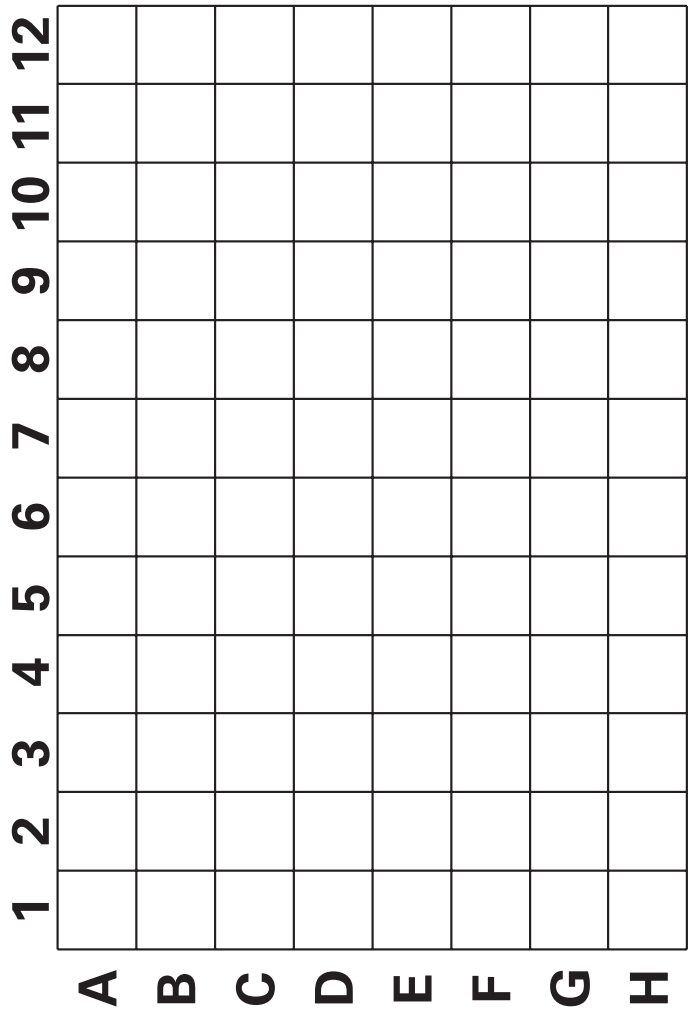
Warranty and Limitation of Remedy

Cayman Chemical Company makes **no warranty or guarantee** of any kind, whether written or oral, expressed or implied, including without limitation, any warranty of fitness for a particular purpose, suitability and merchantability, which extends beyond the description of the chemicals hereof. Cayman **warrants only** to the original customer that the material will meet our specifications at the time of delivery. Cayman will carry out its delivery obligations with due care and skill. Thus, in no event will Cayman have **any obligation or liability**, whether in tort (including negligence) or in contract, for any direct, indirect, incidental or consequential damages, even if Cayman is informed about their possible existence. This limitation of liability does not apply in the case of intentional acts or negligence of Cayman, its directors or its employees.

Buyer's **exclusive remedy** and Cayman's sole liability hereunder shall be limited to a refund of the purchase price, or at Cayman's option, the replacement, at no cost to Buyer, of all material that does not meet our specifications.

Said refund or replacement is conditioned on Buyer giving written notice to Cayman within thirty (30) days after arrival of the material at its destination. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by Buyer of all claims hereunder with respect to said material.

For further details, please refer to our Warranty and Limitation of Remedy located on our website and in our catalog.



NOTES