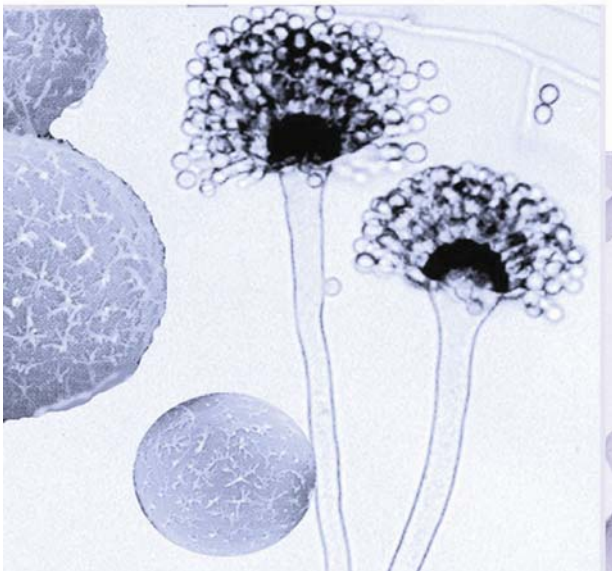
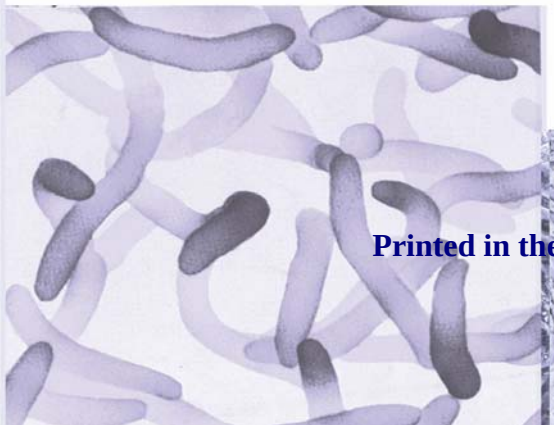
Phenotype MicroArrays™
PM-M TOX1 MicroPlate™

for Measuring Chemosensitivity Phenotypes of Mammalian Cells and for Sensitively Detecting Mitochondrial Toxicity

PRODUCT DESCRIPTION AND INSTRUCTIONS FOR USE
PM-M TOX1, Cat. #14101





PM-M TOX1 MicroPlate™ Biolog Cat. # 14101

<-------- Chemical titration

0x	0.977x	1.953x	3.906x	7.8125x	15.625x	31.25x	62.5x	125x	250x	500x	1000x
A1 α-D-Glucose	A2 α-D-Glucose	A3 α-D-Glucose	A4 α-D-Glucose	A5 α-D-Glucose	A6 α-D-Glucose	A7 α-D-Glucose	A8 α-D-Glucose	A9 α-D-Glucose	A10 α-D-Glucose	A11 α-D-Glucose	A12 α-D-Glucose
B1 Inosine	B2 Inosine	B3 Inosine	B4 Inosine	B5 Inosine	B6 Inosine	B7 Inosine	B8 Inosine	B9 Inosine	B10 Inosine	B11 Inosine	B12 Inosine
C1 D-Galactose	C2 D-Galactose	C3 D-Galactose	C4 D-Galactose	C5 D-Galactose	C6 D-Galactose	C7 D-Galactose	C8 D-Galactose	C9 D-Galactose	C10 D-Galactose	C11 D-Galactose	C12 D-Galactose
D1 D-Glucose-1-Phosphate	D2 D-Glucose-1-Phosphate	D3 D-Glucose-1-Phosphate	D4 D-Glucose-1-Phosphate	D5 D-Glucose-1-Phosphate	D6 D-Glucose-1-Phosphate	D7 D-Glucose-1-Phosphate	D8 D-Glucose-1-Phosphate	D9 D-Glucose-1-Phosphate	D10 D-Glucose-1-Phosphate	D11 D-Glucose-1-Phosphate	D12 D-Glucose-1-Phosphate
E1 Xylitol	E2 Xylitol	E3 Xylitol	E4 Xylitol	E5 Xylitol	E6 Xylitol	E7 Xylitol	E8 Xylitol	E9 Xylitol	E10 Xylitol	E11 Xylitol	E12 Xylitol
F1 α-Keto-Glutaric Acid	F2 α-Keto-Glutaric Acid	F3 α-Keto-Glutaric Acid	F4 α-Keto-Glutaric Acid	F5 α-Keto-Glutaric Acid	F6 α-Keto-Glutaric Acid	F7 α-Keto-Glutaric Acid	F8 α-Keto-Glutaric Acid	F9 α-Keto-Glutaric Acid	F10 α-Keto-Glutaric Acid	F11 α-Keto-Glutaric Acid	F12 α-Keto-Glutaric Acid
G1 D,L-β-Hydroxy-Butyric Acid	G2 D,L-β-Hydroxy-Butyric Acid	G3 D,L-β-Hydroxy-Butyric Acid	G4 D,L-β-Hydroxy-Butyric Acid	G5 D,L-β-Hydroxy-Butyric Acid	G6 D,L-β-Hydroxy-Butyric Acid	G7 D,L-β-Hydroxy-Butyric Acid	G8 D,L-β-Hydroxy-Butyric Acid	G9 D,L-β-Hydroxy-Butyric Acid	G10 D,L-β-Hydroxy-Butyric Acid	G11 D,L-β-Hydroxy-Butyric Acid	G12 D,L-β-Hydroxy-Butyric Acid
H1 Pyruvic Acid	H2 Pyruvic Acid	H3 Pyruvic Acid	H4 Pyruvic Acid	H5 Pyruvic Acid	H6 Pyruvic Acid	H7 Pyruvic Acid	H8 Pyruvic Acid	H9 Pyruvic Acid	H10 Pyruvic Acid	H11 Pyruvic Acid	H12 Pyruvic Acid

I. Product Description

The **PM-M TOX1 MicroPlate** is designed for use as a cell-based assay to examine the effect of a chemical on energy production in a target cell line. It employs a tetrazolium dye chemistry and in that way has similarities to the classical MTT assay. However, the assay is simpler to perform and offers numerous other advantages in sensitivity and specificity. The colorimetric readout of the assays indicates simultaneously, the effect of the chemical on 8 diverse energy production pathways in cells. As such, **it can be used to measure general or mitochondrial toxicity of chemicals as well as inhibition or stimulation of specific energy metabolism pathways in cells**. The MicroPlate has 96 wells. Each of the 8 rows is coated with a different oxidizable carbon source that can be metabolized by most mammalian cells to produce energy. The 12 columns of the MicroPlate can be used for testing a chemical over a 1000-fold concentration range.

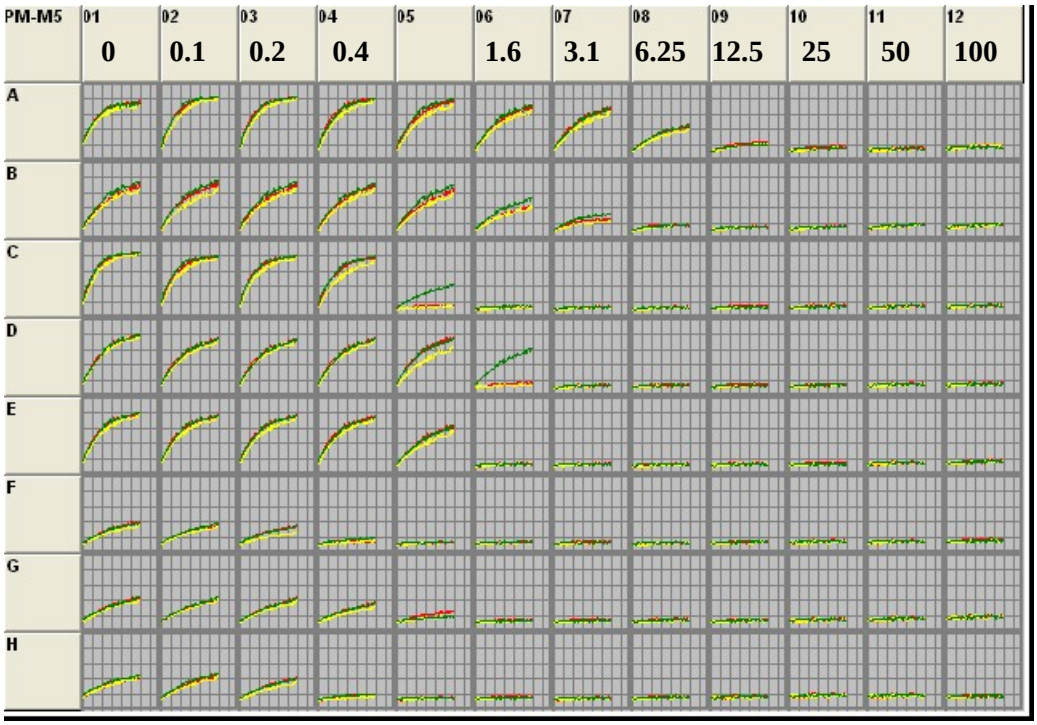
Eight carbon substrates in the PM-M TOX1 MicroPlate

The 8 substrates in the MicroPlate were selected to probe different energetics pathways that various animal cells use to generate NADH. Each substrate is metabolized via one or more different pathways, employing different cellular and mitochondrial transporters and catabolic enzymes. Furthermore, the substrates utilize the cell's mitochondria to different extents and can feed into the electron transport chain at different points. Glucose and galactose are both hexoses, but galactose is metabolized to NADH via mitochondrial action whereas glucose, especially in cancer cells, can bypass mitochondrial functions. This has been used as a basis to detect and distinguish chemicals with mitochondrial toxicity from chemicals with general cytotoxicity (see LD Marroquin, et al., Tox. Sci. (2007) 97:539). Glucose-1-phosphate is metabolized differently from either glucose or galactose. Inosine (which contains ribose) and xylitol are both metabolized via pentose pathways, but in different ways. Of the other substrates, a-ketoglutarate, directly enters the

TCA cycle, whereas b-hydroxybutyrate (a ketone) and pyruvate enter the TCA cycle after metabolism and linkage to coenzyme A. The energetics pathways for the 8 substrates can be different in cells from various tissues.

PM-M TOX1 testing result showing differential sensitivity to FCCP

The Figure below shows an example of titrating the mitochondrial uncoupler FCCP with a liver cancer cell line from 0.1 to 100 uM, with color change recorded kinetically on the Biolog OmniLog instrument. Cells were exposed to FCCP for 48 hours and then Biolog Redox Dye MB containing glucose was added to detect the metabolic activity of cells remaining viable. Note how the cells are much more sensitive to this mitochondrial inhibitor with pyruvate (IC_{50} ~ 0.3 uM) compared with glucose (IC_{50} ~6.25 uM).



Simple testing procedure

The standard testing protocol has 4 simple steps:

- Seed all wells with 2,000 to 20,000 cells and allow the cells 16 hr to adapt.
- Add the chemical to be tested to columns 2 to 12 of the microplate, titrating at 2-fold steps. Typical chemical exposure times could range from several minutes to 48 hours.
- Add Biolog Redox Dye Mix MA or MB (without or with glucose) into the wells. Incubate for about 2 to 6 hr, or until sufficient dye reduction and color formation is observed.
- Detect the metabolic activity or viability of the cells with the 8 different carbon substrates. Measure the reduced dye (formazan) spectrophotometrically at 590 nm (purple color).

An example of a detailed protocol is provided below. Additional information can be found in the Biolog documents "Phenotype MicroArrays™ Panels PM-M1 to PM-M14" and "Biolog Redox Dye Mixes".

II. Example of a Detailed Protocol

a. The materials used are shown below in Table 1.

Table 1. Materials and Equipment for the Testing Procedure

Materials and Equipment	Source	Catalog #
Biolog PM-M TOX1 MicroPlates	Biolog	14101
Biolog Redox Dye Mix MA (6x)	Biolog	74351
Biolog Redox Dye Mix MB (6x)	Biolog	74352
Biolog IF-M1 (1x)	Biolog	72301
D-Glucose (2.5 M)	Sigma	G8769
RPMI 1640 Cell Culture Medium	Invitrogen (or equivalent)	61870
Dulbecco's Phosphate-Buffered Saline (D-PBS) without Mg and Ca	Invitrogen (or equivalent)	14190
Trypsin (0.25%) with EDTA (1 mM)	Invitrogen (or equivalent)	25200-072
Pen/Strep Antibiotic (100x)	Invitrogen (or equivalent)	15070-063
L-Glutamine (200 mM)	Invitrogen (or equivalent)	25030-149
Fetal Bovine Serum (FBS)	Invitrogen (or equivalent)	10082-147
Dialyzed Fetal Bovine Serum (dFBS)	Invitrogen (or equivalent)	26400-036
Trypan Blue Stain (0.4%)	Invitrogen (or equivalent)	15250-061
Cell Strainer, 70 um filters	BD Falcon (or equivalent)	352350
Sterile 75 cm^2 culture flasks	BD Falcon (or equivalent)	353136
Sterile 15 ml conical tubes	BD Falcon (or equivalent)	352096
Sterile 50 ml conical tubes	BD Falcon (or equivalent)	352070
Sterile reservoirs	Biolog (or equivalent)	3102
Multichannel Pipetter	Biolog (or equivalent)	3501A, 3505A and B
Microplate Reader	Biolog (or equivalent)	5044
OmniLog PM Incubator/Reader	Biolog	93171, 93182, 93184

b. Step-by-step protocol.

Step 1 – Seed all wells of the MicroPlate with 2,000 to 20,000 cells and allow the cells 16 hr to adapt.

1) Trypsinize and harvest cells grown in flasks, cultured in RPMI 1640 medium with 10% FBS.
2) Centrifuge and wash the cells with D-PBS.
3) Centrifuge and resuspend the cells in RPMI medium without serum or glucose (IF-M1 + 2mM glutamine + Pen/Strep).
4) Remove cell clumps by filtering cells through a Cell Strainer (70 um filter).
5) Count the number of trypan blue negative cells to determine the density of live cells.
6) Prepare cell suspensions between 50,000 and 500,000 cells/ml in order to seed between 2,000 and 20,000 cells/well, respectively. The exact cell density will depend on the metabolic activity rate of the cells.
7) Dispense **40 ul** of the cell suspension into all wells of prewarmed PM-M TOX1 MicroPlates. For critical assays, plates can be set up and run in duplicate or triplicate.
8) Incubate the MicroPlates overnight (16 hr) at 37°C under 5% CO_2 -95% Air to allow cells to adapt.

Step 2 – Add the chemical to be tested to the MicroPlate.

1) Generate a dilution series by making ten 2-fold dilutions of the chemical to be tested in the same medium used to dispense cells.

2) Add **10 ul** of the chemical dilution series to each of the rows of the PM-M TOX1 MicroPlate.
3) Incubate the MicroPlates for at 37°C under 5% CO_2 -95% Air. Typical chemical exposure times could range from several minutes to 48 hours.

Step 3 – Add Biolog Redox Dye Mix without or with glucose to the MicroPlate.

1) Add **10 ul** of 6x-Biolog Redox Dye Mix MA or MB, without or with 30 mM glucose, to all MicroPlate wells. For substrate metabolism assays, omit the glucose. For cell viability assays, include the glucose. To prepare 6x-Redox Dye Mix with 30 mM glucose, add 0.24 ml of 2.5 M glucose to 20 ml of Redox Dye Mix.
2) Incubate the MicroPlates at 37°C under 5% CO_2 -95% Air until sufficient dye is reduced to generate detectable amounts of purple color. This is typically between 2 and 6 hr depending on the Redox Dye Mix and cell type.

Step 4 – Detect the metabolic activity or viability of the cells with the 8 different carbon substrates.

1) Use a Microplate Reader or a Biolog OmniLog to measure, spectrophotometrically, the amount of reduced dye generated in each well. With a Microplate Reader, the purple formazan can be quantified by measuring the absorbance at 590 nm.

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