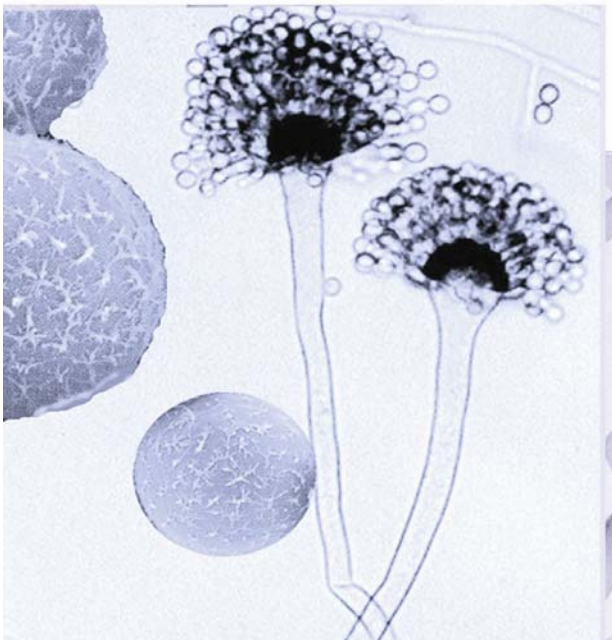
BIOLOG

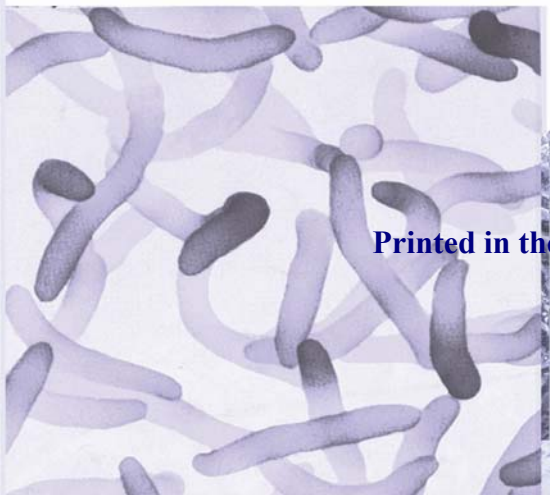
Phenotype MicroArrays™
Panels PM-M1 to PM-M14

for Phenotypic Characterization of Mammalian Cells
Assays: Energy Metabolism Pathways
Ion and Hormone Effects on Cells
Sensitivity to Anti-Cancer Agents
and for Optimizing Culture Conditions for Mammalian Cells

PRODUCT DESCRIPTIONS AND INSTRUCTIONS FOR USE

PM-M1	**Cat. #13101**
PM-M2	**Cat. #13102**
PM-M3	**Cat. #13103**
PM-M4	**Cat. #13104**
PM-M5	**Cat. #13105**
PM-M6	**Cat. #13106**
PM-M7	**Cat. #13107**
PM-M8	**Cat. #13108**
PM-M11	**Cat. #13111**
PM-M12	**Cat. #13112**
PM-M13	**Cat. #13113**
PM-M14	**Cat. #13114**





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Version 02, June 25, 2007. The most current version of this Technical Bulletin can be downloaded from Biolog's website at www.biolog.com. Questions about the use of this product should be directed to Biolog, Inc. Technical Services by E-mail at tech@biolog.com. Phenotype MicroArrays™ and their use are covered by U. S. Patent Nos. 6,436,631, 6,686,173, 6,696,239, and 6,727,076, as well as pending applications, all owned by Biolog, Inc. OmniLog® is a registered trademark of Biolog, Inc. and the OmniLog instrument is covered by U. S. Patent No. 6,271,022, owned by Biolog,Inc.

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I. Introduction

a. Overview

Biolog Phenotype MicroArrays™ (PM-M1 to PM-M4) provide an easy-to-use technology for scanning and measuring the energy metabolism pathways present in a wide range of mammalian cell types from *in vitro* cultured cells to primary cells. The four panels can be used individually or as a set. When the set of 4 PMs is used, 367 potential metabolic pathways are tested simultaneously.

The metabolic pathway activities are assayed with a simple colorimetric reagent that measures redox energy produced when a cell oxidizes a chemical. To perform the assay, a cell suspension is first prepared in an inoculating fluid (IF-M1 or IF-M2) deficient in carbon and energy sources. The suspension is dispensed into the PM wells and incubated for approximately two days during which time the cells adapt to their new environment which includes different carbon and energy sources in the various wells. Then, to measure the cell-mediated metabolism of the chemicals, one of two proprietary color generating systems, Biolog Redox Dye Mix MA or Biolog Redox Dye Mix MB, is added to all wells. The color generating system employs a tetrazolium dye that can be reduced to a purple formazan. Cellular metabolism that is stimulated by the chemical in the well generates reducing equivalents which are captured by the Biolog Redox Dye color generation system.

With appropriately controlled assay conditions, the rate of formazan production is linear with time and can be measured directly in the microwell assay plates without additional processing. Kinetic measurements can be made to determine the rate of formazan production by using Biolog's OmniLog® instrument and software. Alternatively, formazan production can be measured by an endpoint absorbance at 590 nm with a microplate reader.

b. Background

Mammalian cells from various organs and tissues have different capabilities for using substrates and generating energy. They have different metabolic pathway activities which are regulated in different ways by a wide spectrum of chemicals and hormonal signals. PM-M1 to PM–M4 provide a simple tool for the scientist to simultaneously measure 367 of these pathways.

c. Uses

One major use of PMs is in metabolism and nutrition research. PMs can be used to gain a deeper understanding of energy metabolism in virtually any cell line which can also reflect the metabolic properties of the organ or tissue from which the cells were derived. This is essential information in the study of metabolic disorders such as diabetes and obesity, in the study of carbon metabolism and caloric nutrition, and in the testing of drugs, hormones, and any chemical entity that may affect these pathways. Instead of testing one pathway at a time (*e.g.*, only glucose metabolism) the PM panels allow for simultaneous assay of 367 pathways.

A second major use is as a simple tool to fingerprint cells. Different cell lines have different pathway activities so they produce different patterns of purple wells when the PM assay is performed. Furthermore, the scan of 367 metabolic properties reflects the physiological state of the cell. If a cell changes over time, it is likely that its metabolic properties will also change and this can be detected with the sensitive and reproducible PM assays. It is a good practice for everyone working with cell lines to check them weekly, or each time the cells are passaged.

d. Advantages

PM assays are a patented technology that provides a unique and powerful tool for simultaneously testing multiple cellular properties. Some principal advantages are:

- **Proven Technology:** A sizeable published literature documents the successful use of PM assays with microbial cells. An updated listing can be found in the Bibliography section of the Biolog website at www.biolog.com/mID_section_13.html.
- **Simple Protocol:** Add the cells, incubate, add the dye mix, and read.
- **Fast Results:** Sufficient color forms in as little as one hour. The formazan product is soluble and stable in tissue culture medium and can be measured as soon as it forms.
- **Flexible Format:** Either kinetic or endpoint measurements of color development can be performed.
- **Sensitivity:** Low basal rates of dye reduction in the "No Substrate Well" allows for high sensitivity. Cell-mediated metabolic signals can be enhanced by simply extending the incubation time.
- **Broad Applicability:** Tetrazolium reduction assays of mammalian cells have been used for many years in a wide range of applications [1-19]. Biolog's more advanced tetrazolium chemistry [20] also works with a wide range of cell types (e.g., Fig. 1), including liver (HepG2 and C3A), colon (Colo205), lung (A549), prostrate (PC-3), fibroblast (IMR90) and blood (HL-60 and CEM) cells A metabolic fingerprint has also been obtained by direct assay of primary rat liver hepatocytes (e.g., Fig. 2).

II. Product Description, PM-M1 to M4

PM-M1 to PM-M4 are 96-well microplates coated with different oxidizable carbon sources in various wells. The layout of the 367 chemicals is shown in the plate maps on pages that follow. PM-M1 has a range of diverse carbon sources including simple sugars, polysaccharides, and carboxylic acids. PM-M2 to PM-M4 have lipids and protein-derived nutrients, primarily amino acids and dipeptides. The plates are simply warmed to assay temperature and are ready for use.

The standard testing protocol has 4 simple steps:

1. Prepare a cell suspension at 4 x10^5 cells/ml in an appropriate inoculation medium
2. Dispense 50 µl of the cell suspension into the wells of the PM panels and incubate the PM panels for 40 hours at 37° C. in an appropriate atmosphere
3. Dispense 10 µl of Redox Dye Mix into the wells and incubate for 1 to 24 hours until sufficient dye reduction and color formation is observed
4. Measure the reduced dye (formazan) spectrophotometrically

- **Products:** Mammalian PM panels and dyes can be purchased as the following items from Biolog, Inc. or from authorized distributors

Cat. #13101	**Biolog PM-M1**
Cat. #13102	**Biolog PM-M2**
Cat. #13103	**Biolog PM-M3**
Cat. #13104	**Biolog PM-M4**
Cat. #13191	**Biolog PM-M1 to M4 Kit (box of 8 with 2 each)**
Cat. #74351	**Biolog Redox Dye Mix MA (6x)**
Cat. #74352	**Biolog Redox Dye Mix MB (6x)**

- **Intended Use:** For Laboratory Use Only.

- **Biolog PM Storage:** All PM panels should be refrigerated and stored at 4°C. Plates may be taken out and prewarmed to room temperature one day before use. For best results, use before the expiration date printed on the product label.

- **Biolog Redox Dye Mix Storage:** The Dye Mixes must be stored frozen at -20°C and protected from light. They may be repeatedly thawed and used or aliquots can be thawed and kept for one day at 4°C prior to use in assays. For best results, use before the expiration date printed on the product label.

- **Example:** Lung (A459) and liver (C3A) cells exhibit very different metabolic patterns of tetrazolium reduction when incubated in PM panels M1 to M4 (Figure 1).

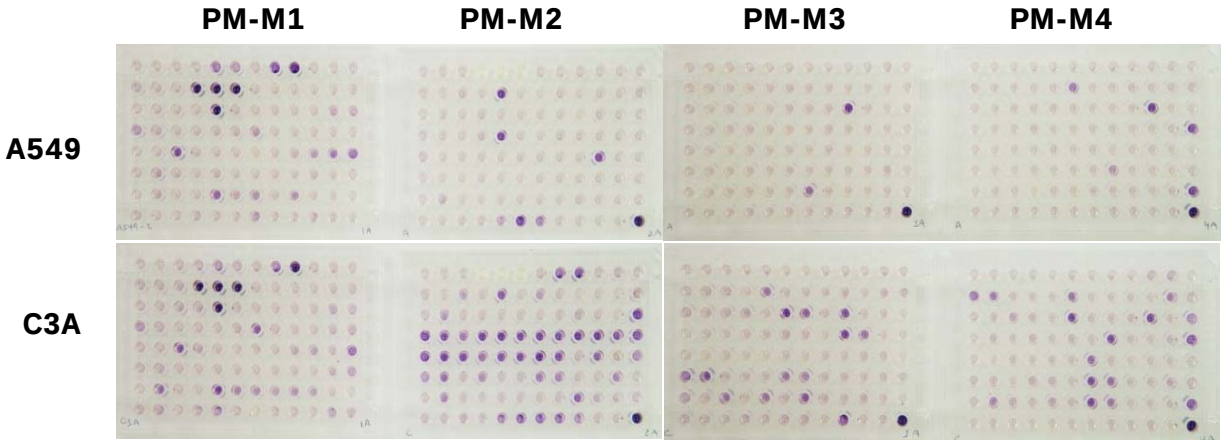

Figure 1. Cultured A549 and C3A cells were harvested by treatment with trypsin, washed in Dulbecco's PBS and suspended at 400,000 cells per ml in Biolog's IF-M1 medium supplemented with 5% FCS, 0.3 mM Gln and 1X Pen Strep. The cells were dispensed into PM panels M1 to M4 (50 µl, 20,000 cells per well) and incubated at 37°C under 5% CO_2 - 95% air for 44 hr. Biolog Redox Dye Mix MA (10 µl) was added to achieve a 1x final concentration and cells were incubated for 2 hr in an OmniLog at 37°C before plates were photographed.

- **PM Panel Maps:** The layout of chemicals in the wells are shown on the pages that follow. Acidic chemicals are the sodium salts and basic chemicals are the chloride salts except as noted: (a): lithium salt, (b): acetate salt, (c): trifluoroacetate salt, (d): bromide salt, (e): formate salt, (f): sulfate salt.

PM-M1 MicroPlate™ - Carbon and Energy Sources

A1 Negative Control	A2 Negative Control	A3 Negative Control	A4 α-Cyclodextrin	A5 Dextrin	A6 Glycogen	A7 Maltitol	A8 Maltotriose	A9 D-Maltose	A10 D-Trehalose	A11 D-Cellobiose	A12 β-Gentiobiose
B1 D-Glucose-6-Phosphate	B2 α-D-Glucose-1-Phosphate	B3 L-Glucose	B4 α-D-Glucose	B5 α-D-Glucose	B6 α-D-Glucose	B7 3-O-Methyl-D-Glucose	B8 α-Methyl-D-Glucoside	B9 β-Methyl-D-Glucoside	B10 D-Salicin	B11 D-Sorbitol	B12 N-Acetyl-D-Glucosamine
C1 D-Glucosaminic Acid	C2 D-Glucuronic Acid	C3 Chondroitin-6-Sulfate	C4 Mannan	C5 D-Mannose	C6 α-Methyl-D-Mannoside	C7 D-Mannitol	C8 N-Acetyl-β-D-Mannosamine	C9 D-Melezitose	C10 Sucrose	C11 Palatinose	C12 D-Turanose
D1 D-Tagatose	D2 L-Sorbose	D3 L-Rhamnose	D4 L-Fucose	D5 D-Fucose	D6 D-Fructose-6-Phosphate	D7 D-Fructose	D8 Stachyose	D9 D-Raffinose	D10 D-Lactitol	D11 Lactulose	D12 α-D-Lactose
E1 Melibionic Acid	E2 D-Melibiose	E3 D-Galactose	E4 α-Methyl-D-Galactoside	E5 β-Methyl-D-Galactoside	E6 N-Acetyl-Neuraminic Acid	E7 Pectin	E8 Sedoheptulosan	E9 Thymidine	E10 Uridine	E11 Adenosine	E12 Inosine
F1 Adonitol	F2 L- Arabinose	F3 D-Arabinose	F4 β-Methyl-D-Xylopyranoside	F5 Xylitol	F6 Myo-Inositol	F7 Meso-Erythritol	F8 Propylene glycol	F9 Ethanolamine	F10 D,L- α-Glycerol-Phosphate	F11 Glycerol	F12 Citric Acid
G1 Tricarballylic Acid	G2 D,L-Lactic Acid	G3 Methyl D-lactate	G4 Methyl pyruvate	G5 Pyruvic Acid	G6 α-Keto-Glutaric Acid	G7 Succinamic Acid	G8 Succinic Acid	G9 Mono-Methyl Succinate	G10 L-Malic Acid	G11 D-Malic Acid	G12 Meso-Tartaric Acid
H1 Acetoacetic Acid (a)	H2 γ-Amino-N-Butyric Acid	H3 α-Keto-Butyric Acid	H4 α-Hydroxy-Butyric Acid	H5 D,L-β-Hydroxy-Butyric Acid	H6 γ-Hydroxy-Butyric Acid	H7 Butyric Acid	H8 2,3-Butanediol	H9 3-Hydroxy-2-Butanone	H10 Propionic Acid	H11 Acetic Acid	H12 Hexanoic Acid

PM-M2 MicroPlate™ - Carbon and Energy Sources / Nitrogen Sources

A1 Negative Control	A2 Negative Control	A3 Negative Control	A4 Tween 20	A5 Tween 40	A6 Tween 80	A7 Gelatin	A8 L-Alaninamide	A9 L-Alanine	A10 D-Alanine	A11 L-Arginine	A12 L-Asparagine
B1 L-Aspartic Acid	B2 D-Aspartic Acid	B3 L-Glutamic Acid	B4 D-Glutamic Acid	B5 L-Glutamine	B6 Glycine	B7 L-Histidine	B8 L-Homoserine	B9 Hydroxy-L-Proline	B10 L-Isoleucine	B11 L-Leucine	B12 L-Lysine
C1 L-Methionine	C2 L-Ornithine	C3 L-Phenylalanine	C4 L-Proline	C5 L-Serine	C6 D-Serine	C7 L-Threonine	C8 D-Threonine	C9 L-Tryptophan	C10 L-Tyrosine	C11 L-Valine	C12 Ala-Ala
D1 Ala-Arg	D2 Ala-Asn	D3 Ala-Asp	D4 Ala-Glu	D5 Ala-Gln	D6 Ala-Gly	D7 Ala-His	D8 Ala-Ile	D9 Ala-Leu	D10 Ala-Lys	D11 Ala-Met	D12 Ala-Phe
E1 Ala-Pro	E2 Ala-Ser	E3 Ala-Thr	E4 Ala-Trp	E5 Ala-Tyr	E6 Ala-Val	E7 Arg-Ala (b)	E8 Arg-Arg (b)	E9 Arg-Asp	E10 Arg-Gln	E11 Arg-Glu	E12 Arg-Ile (b)
F1 Arg-Leu (b)	F2 Arg-Lys (b)	F3 Arg-Met (b)	F4 Arg-Phe (b)	F5 Arg-Ser (b)	F6 Arg-Trp	F7 Arg-Tyr (b)	F8 Arg-Val (b)	F9 Asn-Glu	F10 Asn-Val	F11 Asp-Ala	F12 Asp-Asp
G1 Asp-Glu	G2 Asp-Gln	G3 Asp-Gly	G4 Asp-Leu	G5 Asp-Lys	G6 Asp-Phe	G7 Asp-Trp	G8 Asp-Val	G9 Glu-Ala	G10 Glu-Asp	G11 Glu-Glu	G12 Glu-Gly
H1 Glu-Ser	H2 Glu-Trp	H3 Glu-Tyr	H4 Glu-Val	H5 Gln-Glu	H6 Gln-Gln	H7 Gln-Gly	H8 Gly-Ala	H9 Gly-Arg	H10 Gly-Asn	H11 Gly-Asp	H12 α-D-Glucose

PM-M3 MicroPlate™ - Carbon and Energy Sources / Nitrogen Sources

A1 Negative Control	A2 Negative Control	A3 Negative Control	A4 Gly-Gly	A5 Gly-His	A6 Gly-Ile	A7 Gly-Leu	A8 Gly-Lys	A9 Gly-Met	A10 Gly-Phe	A11 Gly-Pro	A12 Gly-Ser
B1 Gly-Thr	B2 Gly-Trp	B3 Gly-Tyr	B4 Gly-Val	B5 His-Ala	B6 His-Asp	B7 His-Glu	B8 His-Gly	B9 His-His (c)	B10 His-Leu	B11 His-Lys (d)	B12 His-Met
C1 His-Pro	C2 His-Ser	C3 His-Trp	C4 His-Tyr	C5 His-Val	C6 Ile-Ala	C7 Ile-Arg (b)	C8 Ile-Asn	C9 Ile-Gln	C10 Ile-Gly	C11 Ile-His	C12 Ile-Ile
D1 Ile-Leu	D2 Ile-Met	D3 Ile-Phe	D4 Ile-Pro	D5 Ile-Ser	D6 Ile-Trp	D7 Ile-Tyr	D8 Ile-Val	D9 Leu-Ala	D10 Leu-Arg (b)	D11 Leu-Asn	D12 Leu-Asp
E1 Leu-Glu	E2 Leu-Gly	E3 Leu-His	E4 Leu-Ile	E5 Leu-Leu	E6 Leu-Met	E7 Leu-Phe	E8 Leu-Pro	E9 Leu-Ser	E10 Leu-Trp	E11 Leu-Tyr	E12 Leu-Val
F1 Lys-Ala (d)	F2 Lys-Arg (b)	F3 Lys-Asp	F4 Lys-Glu	F5 Lys-Gly	F6 Lys-Ile (b)	F7 Lys-Leu (b)	F8 Lys-Lys	F9 Lys-Met (e)	F10 Lys-Phe	F11 Lys-Pro	F12 Lys-Ser
G1 Lys-Thr	G2 Lys-Trp (b)	G3 Lys-Tyr (b)	G4 Lys-Val (d)	G5 Met-Arg (b)	G6 Met-Asp	G7 Met-Gln	G8 Met-Glu	G9 Met-Gly	G10 Met-His	G11 Met-Ile	G12 Met-Leu
H1 Met-Lys (e)	H2 Met-Met	H3 Met-Phe	H4 Met-Pro	H5 Met-Thr	H6 Met-Trp	H7 Met-Tyr	H8 Met-Val	H9 Phe-Ala	H10 Phe-Asp	H11 Phe-Glu	H12 α-D-Glucose

PM-M4 MicroPlate™ - Carbon and Energy Sources / Nitrogen Sources

A1 Negative Control	A2 Negative Control	A3 Negative Control	A4 Phe-Gly	A5 Phe-Ile	A6 Phe-Met	A7 Phe-Phe	A8 Phe-Pro	A9 Phe-Ser	A10 Phe-Trp	A11 Phe-Tyr	A12 Phe-Val
B1 Pro-Ala	B2 Pro-Arg (b)	B3 Pro-Asn	B4 Pro-Asp	B5 Pro-Glu	B6 Pro-Gln	B7 Pro-Gly	B8 Pro-Hyp	B9 Pro-Ile	B10 Pro-Leu	B11 Pro-Lys (b)	B12 Pro-Phe
C1 Pro-Pro	C2 Pro-Ser	C3 Pro-Trp	C4 Pro-Tyr	C5 Pro-Val	C6 Ser-Ala	C7 Ser-Asn	C8 Ser-Asp	C9 Ser-Glu	C10 Ser-Gln	C11 Ser-Gly	C12 Ser-His (b)
D1 Ser-Leu	D2 Ser-Met	D3 Ser-Phe	D4 Ser-Pro	D5 Ser-Ser	D6 Ser-Tyr	D7 Ser-Val	D8 Thr-Ala	D9 Thr-Arg (f)	D10 Thr-Asp	D11 Thr-Glu	D12 Thr-Gln
E1 Thr-Gly	E2 Thr-Leu	E3 Thr-Met	E4 Thr-Phe	E5 Thr-Pro	E6 Thr-Ser	E7 Trp-Ala	E8 Trp-Arg	E9 Trp-Asp	E10 Trp-Glu	E11 Trp-Gly	E12 Trp-Leu
F1 Trp-Lys (e)	F2 Trp-Phe	F3 Trp-Ser	F4 Trp-Trp	F5 Trp-Tyr	F6 Trp-Val	F7 Tyr-Ala	F8 Tyr-Gln	F9 Tyr-Glu	F10 Tyr-Gly	F11 Tyr-His	F12 Tyr-Ile
G1 Tyr-Leu	G2 Tyr-Lys	G3 Tyr-Phe	G4 Tyr-Trp	G5 Tyr-Tyr	G6 Tyr-Val	G7 Val-Ala	G8 Val-Arg	G9 Val-Asn	G10 Val-Asp	G11 Val-Glu	G12 Val-Gln
H1 Val-Gly	H2 Val-His	H3 Val-Ile	H4 Val-Leu	H5 Val-Lys	H6 Val-Met	H7 Val-Phe	H8 Val-Pro	H9 Val-Ser	H10 Val-Tyr	H11 Val-Val	H12 α-D-Glucose

III. Protocols, PM-M1 to M4

a. Materials Required

Table 1. Equipment

Equipment	Source	Catalog #
OmniLog PM System	Biolog	93171, 93182, 93184
Microplate Reader	Biolog (or equivalent)	5044
Multichannel Pipetter	Biolog	3711

Table 2. Chemicals and Materials for Inoculation Procedure

Chemicals and Materials	Source	Catalog #
PM panels (PM-M1 through PM-M4)	Biolog	13101, 13102, 13103, 13104 13191 (kit of 2 each)
Biolog Redox Dye Mix MA (6x)	Biolog	74351
Biolog Redox Dye Mix MB (6x)	Biolog	74352
Biolog IF-M1 (1x)	Biolog	72301
Biolog IF-M2 (1x)	Biolog	72302
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
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	3102
Sterile sealing tape for 96-well plates	Sigma	Z369667

b. Determination of Which Redox Dye Mix to Use

Biolog provides two Redox Dye Mixes to cover a very wide range of cell types. Redox Dye Mix MA enables color generation in 1 to 6 hr with most cell lines, including liver (HepG2 and C3A), colon (Colo205), lung (A549) and prostate (PC-3) cells. Redox Dye Mix MB enables color generation in 5 to 24 hr with fibroblasts (IMR90) and blood cells (HL-60 and CEM). When starting out with a new cell line, we recommend that you evaluate both Redox Dye Mixes. Perform a side-by-side comparison under identical PM assay conditions and select the Redox Dye Mix that produces the greatest number of wells showing purple formazan.

c. Preparation of Inoculating Medium

Prepare the standard PM assay medium by adding the following to a bottle containing 100 ml of Biolog IF-M1: 1.1 ml of 100x Pen/Strep solution, 0.16 ml of 200 mM Glutamine (final concentration 0.3 mM), and 5.3 ml of FBS/FCS (final concentration 5%). Mix thoroughly. This medium is referred to as complete MC-0 Assay Medium.

d. Preparation of Cell Suspensions

1. Prepare an adequate supply of healthy, growing cells for testing by culturing them in a 75 cm^2 culture flask using an appropriate culture medium such as RPMI with 10% Fetal Bovine Serum. Prepare complete MC-0 Assay Medium and prewarm it to 37°C. Have the D-PBS and the Trypsin with EDTA solution (pre-diluted 1 to 1 with D-PBS) at room temperature. If you are working with a non-adherent cell line, skip the cell detachment steps 2 through 4 and go to step 5.

2. Remove 10 ml of medium from the culture flask and save it in a 15 ml sterile conical tube. Aspirate and discard the remaining medium from the culture flask. Wash the adherent cells twice with 10 ml of D-PBS and aspirate and discard any remaining D-PBS.

3. Detach the cells by treating with Trypsin. Add 2 ml of Trypsin with EDTA (pre-diluted 1 to 1 with D-PBS) to cover the cell monolayer in the culture flask and incubate at 37°C until the cells just detach from the surface. This typically takes about 2 minutes.

4. Quench the detachment reaction by adding 3 ml of culture medium taken from the 15 ml conical tube (or trypsin inhibitor mixture) and mix the cell suspension by gently pipetting up and down several times to disperse the cells.

5. Harvest the cells by transferring the cell suspension to the 15 ml conical tube containing the culture medium (or no culture medium if cells are non-adherent) and centrifuge at 350 x g for 10 minutes. After centrifugation, aspirate the medium and add 10 ml of D-PBS. Suspend the cell pellet in the D-PBS by pipetting up and down several times, then centrifuge again at 350 x g for 10 minutes.

6. After the second centrifugation, aspirate the medium and add 10 ml of pre-warmed MC-0. Suspend the cell pellet in the MC-0 Assay Medium by pipetting up and down several times.

7. Determine cell number and check cell viability using trypan blue exclusion. Remove 90 µl of the cell suspension and add 10 µl of trypan blue. Place the mixture in a hemocytometer and count the total number of cells and total number of blue cells. Do not continue if the non-viable trypan blue positive cells is greater than >10% of the total number.

8. Suspend the cells in enough MC-0 Assay Medium to fill the selected number of PM panels and to achieve a density of 4 $x10^5$ cells/ml. **Note**: Each PM plate will require 5 ml of MC-0 Assay Medium plus an additional 2 ml to have enough volume in the sterile reservoir for dispensing (For four PM-M plates, this will translate to 22 ml).

e. Inoculation and Incubation

1. Transfer the cell suspension into a sterile reservoir and, using a multichannel pipetter, add 50 µl/well of the cell suspension to the plate so that each well has 20,000 cells. Work quickly and mix the cell suspension occasionally to ensure that cells do not settle while dispensing them into the PM wells.

2. Incubate the PM plates at 37°C in a humidified atmosphere with 95% Air-5% CO_2 for 40 to 48 hours. **Note:** You can select a time within this range that is convenient for your work schedule but the incubation time should be consistent to maintain reproducible assay results.

f. Dye Addition and Color Development

1. Add the Biolog Redox Dye Mix to all wells. Transfer the Dye Mix to a sterile reservoir and, using a multichannel pipetter, add 10 µl/well to the plate. Seal the plate with tape to prevent off-gassing of CO_2. **Note:** The Redox Dye Mix added should have been determined in preliminary experiments.

2. Incubate until sufficient color develops. This is typically 1 to 6 hr with Biolog Redox Dye Mix MA and 5 to 24 hr with Biolog Redox Dye Mix MB. Only a few cell lines, such as blood cells, are slow to reduce the tetrazolium and may need 24 hours. **Note:** Absorbance measurements can be performed at a later time by stopping the bio-reduction with SDS. Add about 2% SDS (e.g., 15 µl of 10% SDS to 60 µl of cell culture/dye mixture) to each well to stop the reaction. Store SDS-treated plates in a humid environment and protected from light at room temperature for up to 18 hours.

g. Reading and Quantitation of Results

1. Tetrazolium reduction can be measured kinetically using Biolog's OmniLog PM instrument which offers several advantages. (1) Up to 50 microplates can be read concurrently. (2) A kinetic readout of color formation can be obtained. (3) Rates of tetrazolium reduction can be determined. (4) Temperature can be changed to examine its effect on metabolism. An example of an OmniLog kinetic readout for 2 hours is shown in Figure 2.a. Kinetics are linear for approximately 60 minutes. Accurate kinetic rate measurements are obtained using early time points and only accepting OmniLog values below 200 since the readings become non-linear at higher values.

2. Tetrazolium reduction at fixed time endpoints can also be determined with a Microplate Reader to evaluate the Absorbance at 590 nm (A_{590}). The endpoint reads can also be performed at 590 nm with subtraction of a 750 nm reference reading ($A_{590-750}$) which corrects for any background light scattering. A picture of a PM-M1 plate photographed after a 2 hour endpoint assay is shown in Figure 2.b.

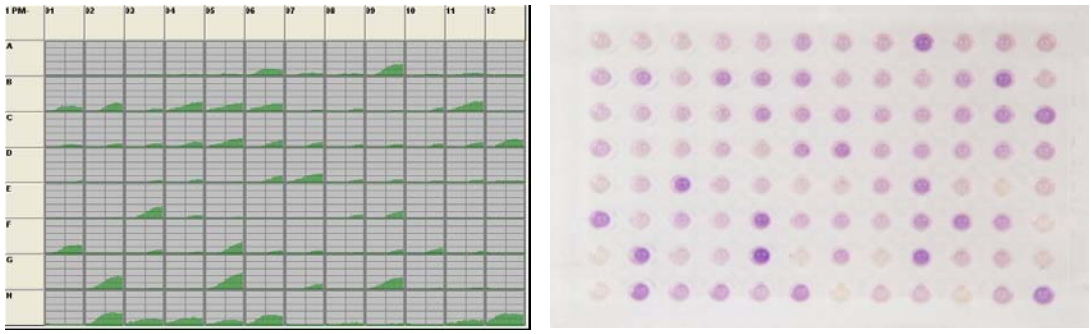

Figure 2. Primary rat liver hepatocytes assayed in PM-M1 for 6 hr. Results are shown (a.) kinetically, as monitored by the OmniLog, with subtraction of the background color in the negative control wells, and (b.) at the endpoint of incubation.

h. Variations of the Standard Protocol

Because metabolic pathways are regulated, the activity of pathways in a cell can change with varying assay conditions. For research applications, the PM assay conditions can be varied to help determine the range of cellular energy-producing pathways that can be activated in a cell line and, at the same time determine the effect of relevant factors such as hormones and chemicals, on these pathways. For cell line fingerprinting, one can employ variations of the protocol to expand the range of tests and obtain a more detailed and precise view of the cell's metabolic properties.

We have found 3 variations of the cell suspension preparation step that can often induce significant changes in the metabolic patterns. These can be useful for obtaining different pathway responses from a given cell line:

1. Substitute dialyzed serum (dFBS) for regular FBS.
2. Alter the serum concentration from 0% to 15 or 20%.
3. Substitute Biolog IF-M2 which lacks all 20 amino acids for Biolog IF-M1.

To get an extremely detailed characterization of a cell line, it could be tested with both inoculating fluids and 5 variations of serum content, as shown below, expanding the number of assays 10-fold:

IF-M1 (+aa)	0% FBS	5% FBS	5% dFBS	15% FBS	15% dFBS
IF-M2 (-aa)	0% FBS	5% FBS	5% dFBS	15% FBS	15% dFBS

The variations can also be combined and performed as a titration. For example, to titrate the amino acids, experiments can be performed with media obtained by mixing different proportions of IF-M2 (which lacks all 20 amino acids) with IF-M1 (which contains amino acids). Other experiments can be performed mixing FBS and dFBS, titrating the total serum concentration, *etc*. An example of altered metabolic activities induced by substituting dFBS for FBS is shown in Figure 3 below.

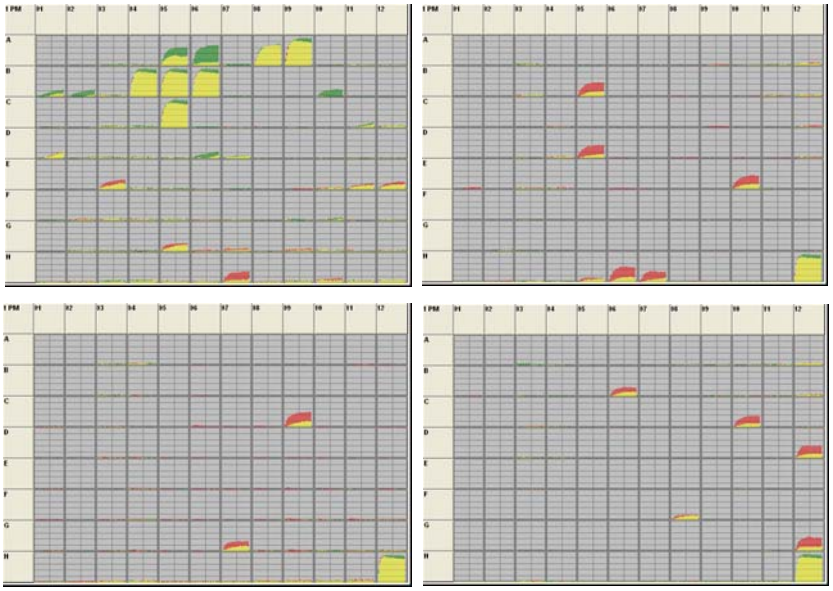

Figure 3. Changes in the metabolism of lung A549 cells induced by altering the serum content of the assay medium. Cells were assayed with the standard protocol and data was collected for 6 hr using the OmniLog and PM software, with subtraction of the background color in the negative control wells. Metabolic responses are plotted for the 367 substrates in PM-M1 (top left), PM-M2 (top right), PM-M3 (bottom left) and PM-M4 (bottom right). Results obtained using the standard medium (IF-M1 containing 0.3 mM Gln and 5% FBS) are shown in red and results obtained with dFBS replacing FBS (i.e., Variation 1) are shown in green. Yellow color indicates responses that are overlapping in the two assay conditions. In PM-M1, Variation 1 resulted in increased metabolism (green color) of glycogen (A-6), dextrin (A-5), salicin (B-10), G1P (B-2), G6P (B-1), and F6P (D-6) and decreased metabolism (red color) of galactose (E-3), pyruvate (G-5), butyrate (H-7). Also metabolism of glutamine (B-5 in PM-M2) and a number of glutamine dipeptides in PM-M2, -M3, and -M4 was reduced in the presence of dFBS.

IV. Product Description, PM-M5 to M14

PM-M5 to PM-M8 are 96-well microplates coated with different ions, hormones, and other metabolic effectors. PM-M11 to PM-M14 are 96-well microplates coated with different anti-cancer agents. The layout of the chemicals is shown in the plate maps on pages that follow. Each chemical is titrated at 4 or 6 increasing concentrations from left to right in the sequence of wells. The plates are simply warmed to assay temperature and are ready for use.

- **Products:** Mammalian PM panels and dyes can be purchased as the following items from Biolog, Inc. or from authorized distributors

Cat. #13105	**Biolog PM-M5**
Cat. #13106	**Biolog PM-M6**
Cat. #13107	**Biolog PM-M7**
Cat. #13108	**Biolog PM-M8**
Cat. #13111	**Biolog PM-M11**
Cat. #13112	**Biolog PM-M12**
Cat. #13113	**Biolog PM-M13**
Cat. #13114	**Biolog PM-M14**
Cat. #74351	**Biolog Redox Dye Mix MA (6x)**
Cat. #74352	**Biolog Redox Dye Mix MB (6x)**

- **Intended Use:** For Laboratory Use Only.

- **Biolog PM Storage:** All PM panels should be refrigerated and stored at 4^{o}C. Plates may be taken out and prewarmed to room temperature one day before use. For best results, use before the expiration date printed on the product label.

PM-M5 MicroPlate™ - Ions

A1 Negative Control	A2 Negative Control	A3 Negative Control	A4 Negative Control	A5 NaCl 1	A6 NaCl 2	A7 NaCl 3	A8 NaCl 4	A9 NaCl 5	A10 NaCl 6	A11 NaCl 7	A12 NaCl 8
B1 Ammonium Chloride 1	B2 Ammonium Chloride 2	B3 Ammonium Chloride 3	B4 Ammonium Chloride 4	B5 Sodium Selenite 1	B6 Sodium Selenite 2	B7 Sodium Selenite 3	B8 Sodium Selenite 4	B9 Potassium Chloride 1	B10 Potassium Chloride 2	B11 Potassium Chloride 3	B12 Potassium Chloride 4
C1 Calcium Chloride 1	C2 Calcium Chloride 2	C3 Calcium Chloride 3	C4 Calcium Chloride 4	C5 Manganese Chloride 1	C6 Manganese Chloride 2	C7 Manganese Chloride 3	C8 Manganese Chloride 4	C9 Zinc Chloride 1	C10 Zinc Chloride 2	C11 Zinc Chloride 3	C12 Zinc Chloride 4
D1 Copper (II) Chloride 1	D2 Copper (II) Chloride 2	D3 Copper (II) Chloride 3	D4 Copper (II) Chloride 4	D5 Cobalt Chloride 1	D6 Cobalt Chloride 2	D7 Cobalt Chloride 3	D8 Cobalt Chloride 4	D9 Iodine 1	D10 Iodine 2	D11 Iodine 3	D12 Iodine 4
E1 Sodium Phosphate 1	E2 Sodium Phosphate 2	E3 Sodium Phosphate 3	E4 Sodium Phosphate 4	E5 Sodium Sulfate 1	E6 Sodium Sulfate 2	E7 Sodium Sulfate 3	E8 Sodium Sulfate 4	E9 Sodium Molybdate 1	E10 Sodium Molybdate 2	E11 Sodium Molybdate 3	E12 Sodium Molybdate 4
F1 Sodium Tungstate 1	F2 Sodium Tungstate 2	F3 Sodium Tungstate 3	F4 Sodium Tungstate 4	F5 Sodium Orthovanadate 1	F6 Sodium Orthovanadate 2	F7 Sodium Orthovanadate 3	F8 Sodium Orthovanadate 4	F9 Potassium Chromate 1	F10 Potassium Chromate 2	F11 Potassium Chromate 3	F12 Potassium Chromate 4
G1 Sodium Pyrophosphate 1	G2 Sodium Pyrophosphate 2	G3 Sodium Pyrophosphate 3	G4 Sodium Pyrophosphate 4	G5 Sodium Nitrate 1	G6 Sodium Nitrate 2	G7 Sodium Nitrate 3	G8 Sodium Nitrate 4	G9 Sodium Nitrite 1	G10 Sodium Nitrite 2	G11 Sodium Nitrite 3	G12 Sodium Nitrite 4
H1 Lithium Chloride 1	H2 Lithium Chloride 2	H3 Lithium Chloride 3	H4 Lithium Chloride 4	H5 Ferric Chloride 1	H6 Ferric Chloride 2	H7 Ferric Chloride 3	H8 Ferric Chloride 4	H9 Magnesium Chloride 1	H10 Magnesium Chloride 2	H11 Magnesium Chloride 3	H12 Magnesium Chloride 4

PM-M6 MicroPlate™ - Hormones & Metabolic Effectors

A1 Negative Control	A2 Negative Control	A3 Negative Control	A4 Negative Control	A5 Negative Control	A6 Negative Control	A7 dibutyryl-cAMP 1	A8 dibutyryl-cAMP 2	A9 dibutyryl-cAMP 3	A10 dibutyryl-cAMP 4	A11 dibutyryl-cAMP 5	A12 dibutyryl-cAMP 6
B1 3-Isobutyl-1-methylxanthine 1	B2 3-Isobutyl-1-methylxanthine 2	B3 3-Isobutyl-1-methylxanthine 3	B4 3-Isobutyl-1-methylxanthine 4	B5 3-Isobutyl-1-methylxanthine 5	B6 3-Isobutyl-1-methylxanthine 6	B7 Caffeine 1	B8 Caffeine 2	B9 Caffeine 3	B10 Caffeine 4	B11 Caffeine 5	B12 Caffeine 6
C1 Epinephrine 1	C2 Epinephrine 2	C3 Epinephrine 3	C4 Epinephrine 4	C5 Epinephrine 5	C6 Epinephrine 6	C7 Norepinephrine 1	C8 Norepinephrine 2	C9 Norepinephrine 3	C10 Norepinephrine 4	C11 Norepinephrine 5	C12 Norepinephrine 6
D1 L-Leucine 1	D2 L-Leucine 2	D3 L-Leucine 3	D4 L-Leucine 4	D5 L-Leucine 5	D6 L-Leucine 6	D7 Creatine 1	D8 Creatine 2	C9 Dreatine 3	D10 Creatine 4	D11 Creatine 5	D12 Creatine 6
E1 Triiodothyronine 1	E2 Triiodothyronine 2	E3 Triiodothyronine 3	E4 Triiodothyronine 4	E5 Triiodothyronine 5	E6 Triiodothyronine 6	E7 Thyroxine 1	E8 Thyroxine 2	E9 Thyroxine 3	E10 Thyroxine 4	E11 Thyroxine 5	E12 Thyroxine 6
F1 Dexamethasone 1	F2 Dexamethasone 2	F3 Dexamethasone 3	F4 Dexamethasone 4	F5 Dexamethasone 5	F6 Dexamethasone 6	F7 Hydrocortisone 1	F8 Hydrocortisone 2	F9 Hydrocortisone 3	F10 Hydrocortisone 4	F11 Hydrocortisone 5	F12 Hydrocortisone 6
G1 Progesterone 1	G2 Progesterone 2	G3 Progesterone 3	G4 Progesterone 4	G5 Progesterone 5	G6 Progesterone 6	G7 β –Estradiol 1	G8 β –Estradiol 2	G9 β –Estradiol 3	G10 β –Estradiol 4	G11 β –Estradiol 5	G12 β –Estradiol 6
H1 4,5α-Dihydro-testosterone 1	H2 4,5α-Dihydro-testosterone 2	H3 4,5α-Dihydro-testosterone 3	H4 4,5α-Dihydro-testosterone 4	H5 4,5α-Dihydro-testosterone 5	H6 4,5α-Dihydro-testosterone 6	H7 Aldosterone 1	H8 Aldosterone 2	H9 Aldosterone 3	H10 Aldosterone 4	H11 Aldosterone 5	H12 Aldosterone 6

PM-M7 MicroPlate™ - Hormones & Metabolic Effectors

A1 Negative Control	A2 Negative Control	A3 Negative Control	A4 Negative Control	A5 Negative Control	A6 Negative Control	A7 Insulin 1	A8 Insulin 2	A9 Insulin 3	A10 Insulin 4	A11 Insulin 5	A12 Insulin 6
B1 Resistin 1	B2 Resistin 2	B3 Resistin 3	B4 Resistin 4	B5 Resistin 5	B6 Resistin 6	B7 Glucagon 1	B8 Glucagon 2	B9 Glucagon 3	B10 Glucagon 4	B11 Glucagon 5	B12 Glucagon 6
C1 Ghrelin 1	C2 Ghrelin 2	C3 Ghrelin 3	C4 Ghrelin 4	C5 Ghrelin 5	C6 Ghrelin 6	C7 Leptin 1	C8 Leptin 2	C9 Leptin 3	C10 Leptin 4	C11 Leptin 5	C12 Leptin 6
D1 Gastrin 1	D2 Gastrin 2	D3 Gastrin 3	D4 Gastrin 4	D5 Gastrin 5	D6 Gastrin 6	D7 Exendin-3 1	D8 Exendin-3 2	D9 Exendin-3 3	D10 Exendin-3 4	D11 Exendin-3 5	D12 Exendin-3 6
E1 hGH (Somatotropin) 1	E2 hGH (Somatotropin) 2	E3 hGH (Somatotropin) 3	E4 hGH (Somatotropin) 4	E5 hGH (Somatotropin) 5	E6 hGH (Somatotropin) 6	E7 IGF-I 1	E8 IGF-I 2	E9 IGF-I 3	E10 IGF-I 4	E11 IGF-I 5	E12 IGF-I 6
F1 FGF-1 (aFGF) 1	F2 FGF-1 (aFGF) 2	F3 FGF-1 (aFGF) 3	F4 FGF-1 (aFGF) 4	F5 FGF-1 (aFGF) 5	F6 FGF-1 (aFGF) 6	F7 PDGF-AB 1	F8 PDGF-AB 2	F9 PDGF-AB 3	F10 PDGF-AB 4	F11 PDGF-AB 5	F12 PDGF-AB 6
G1 IL-1β 1	G2 IL-1β 2	G3 IL-1β 3	G4 IL-1β 4	G5 IL-1β 5	G6 IL-1β 6	G7 IL-2 1	G8 IL-2 2	G9 IL-2 3	G10 IL-2 4	G11 IL-2 5	G12 IL-2 6
H1 IL-6 1	H2 IL-6 2	H3 IL-6 3	H4 IL-6 4	H5 IL-6 5	H6 IL-6 6	H7 IL-8 1	H8 IL-8 2	H9 IL-8 3	H10 IL-8 4	H11 IL-8 5	H12 IL-8 6

PM-M8 MicroPlate™ - Hormones & Metabolic Effectors

A1 Negative Control	A2 Negative Control	A3 Negative Control	A4 Negative Control	A5 Negative Control	A6 Negative Control	A7 (Arg8) - Vasopressin 1	A8 (Arg8) - Vasopressin 2	A9 (Arg8) - Vasopressin 3	A10 (Arg8) - Vasopressin 4	A11 (Arg8) - Vasopressin 5	A12 (Arg8) - Vasopressin 6
B1 Parathyroid Hormone 1	B2 Parathyroid Hormone 2	B3 Parathyroid Hormone 3	B4 Parathyroid Hormone 4	B5 Parathyroid Hormone 5	B6 Parathyroid Hormone 6	B7 Prolactin 1	B8 Prolactin 2	B9 Prolactin 3	B10 Prolactin 4	B11 Prolactin 5	B12 Prolactin 6
C1 Calcitonin 1	C2 Calcitonin 2	C3 Calcitonin 3	C4 Calcitonin 4	C5 Calcitonin 5	C6 Calcitonin 6	C7 Calcitriol (1α,25-Dihydroxyvitamin D3) 1	C8 Calcitriol (1α,25-Dihydroxyvitamin D3) 2	C9 Calcitriol (1α,25-Dihydroxyvitamin D3) 3	C10 Calcitriol (1α,25-Dihydroxyvitamin D3) 4	C11 Calcitriol (1α,25-Dihydroxyvitamin D3) 5	C12 Calcitriol (1α,25-Dihydroxyvitamin D3) 6
D1 Luteinizing hormone (LH) 1	D2 Luteinizing hormone (LH) 2	D3 Luteinizing hormone (LH) 3l	D4 Luteinizing hormone (LH) 4	D5 Luteinizing hormone (LH) 5	D6 Luteinizing hormone (LH) 6	D7 Luteinizing hormone releasing hormone (LH-RH) 1	D8 Luteinizing hormone releasing hormone (LH-RH) 2	D9 Luteinizing hormone releasing hormone (LH-RH) 3	D10 Luteinizing hormone releasing hormone (LH-RH) 4	D11 Luteinizing hormone releasing hormone (LH-RH) 5	D12 Luteinizing hormone releasing hormone (LH-RH) 6
E1 Chorionic gonadotropin human (HCG) 1	E2 Chorionic gonadotropin human (HCG) 2	E3 Chorionic gonadotropin human (HCG) 3	E4 Chorionic gonadotropin human (HCG) 4	E5 Chorionic gonadotropin human (HCG) 5	E6 Chorionic gonadotropin human (HCG) 6	E7 Adrenocortico-tropic hormone human (ACTH) 1	E8 Adrenocortico-tropic hormone human (ACTH) 2	E9 Adrenocortico-tropic hormone human (ACTH) 3	E10 Adrenocortico-tropic hormone human (ACTH) 4	E11 Adrenocortico-tropic hormone human (ACTH) 5	E12 Adrenocortico-tropic hormone human (ACTH) 6
F1 Thyrotropic hormone (TSH) 1	F2 Thyrotropic hormone (TSH) 2	F3 Thyrotropic hormone (TSH) 3	F4 Thyrotropic hormone (TSH) 4	F5 Thyrotropic hormone (TSH) 5	F6 Thyrotropic hormone (TSH) 6	F7 Thyrotropin releasing hormone acetate salt (TRH) 1	F8 Thyrotropin releasing hormone acetate salt (TRH) 2	F9 Thyrotropin releasing hormone acetate salt (TRH) 3	F10 Thyrotropin releasing hormone acetate salt (TRH) 4	F11 Thyrotropin releasing hormone acetate salt (TRH) 5	F12 Thyrotropin releasing hormone acetate salt (TRH) 6
G1 IFN-γ 1	G2 IFN-γ 2l	G3 IFN-γ 3	G4 IFN-γ 4	G5 IFN-γ 5	G6 IFN-γ 6	G7 TNF-α 1	G8 TNF-α 2	G9 TNF-α 3	G10 TNF-α 4	G11 TNF-α 5	G12 TNF-α 6
H1 Adenosine 1	H2 Adenosine 2	H3 Adenosine 3	H4 Adenosine 4	H5 Adenosine 5	H6 Adenosine 6	H7 Gly-His-Lys acetate salt 1	H8 Gly-His-Lys acetate salt 2	H9 Gly-His-Lys acetate salt 3	H10 Gly-His-Lys acetate salt 4	H11 Gly-His-Lys acetate salt 5	H12 Gly-His-Lys acetate salt 6

PM-M11 MicroPlate™ - Anti-Cancer Agents

A1 Negative Control	A2 Negative Control	A3 Negative Control	A4 Negative Control	A5 Solasodine 1	A6 Solasodine 2	A7 Solasodine 3	A8 Solasodine 4	A9 Rotenone 1	A10 Rotenone 2	A11 Rotenone 3	A12 Rotenone 4
B1 Aklavine Hydrochloride 1	B2 Aklavine Hydrochloride 2	B3 Aklavine Hydrochloride 3	B4 Aklavine Hydrochloride 4	B5 Deguelin(-) 1	B6 Deguelin(-) 2	B7 Deguelin(-) 3	B8 Deguelin(-) 4	B9 Celastrol 1	B10 Celastrol 2	B11 Celastrol 3	B12 Celastrol 4
C1 Juglone 1	C2 Juglone 2	C3 Juglone 3	C4 Juglone 4	C5 Sanguinarine Sulfate 1	C6 Sanguinarine Sulfate 2	C7 Sanguinarine Sulfate 3	C8 Sanguinarine Sulfate 4	C9 Dactinomycin 1	C10 Dactinomycin 2	C11 Dactinomycin 3	C12 Dactinomycin 4
D1 Methylmethane Sulfonate 1	D2 Methylmethane Sulfonate 2	D3 Methylmethane Sulfonate 3	D4 Methylmethane Sulfonate 4	D5 Azathioprine 1	D6 Azathioprine 2	D7 Azathioprine 3	D8 Azathioprine 4	D9 Busulfan 1	D10 Busulfan 2	D11 Busulfan 3	D12 Busulfan 4
E1 Aclarubicin 1	E2 Aclarubicin 2	E3 Aclarubicin 3	E4 Aclarubicin 4	E5 Chloramphenicol 1	E6 Chloramphenicol 2	E7 Chloramphenicol 3	E8 Chloramphenicol 4	E9 Chloroquine Diphosphate 1	E10 Chloroquine Diphosphate 2	E11 Chloroquine Diphosphate 3	E12 Chloroquine Diphosphate 4
F1 Cyclophosphamide 1	F2 Cyclophosphamide 2	F3 Cyclophosphamide 3	F4 Cyclophosphamide 4	F5 Diethylcarbamazine Citrate 1	F6 Diethylcarbamazine Citrate 2	F7 Diethylcarbamazine Citrate 3	F8 Diethylcarbamazine Citrate 4	F9 Emetine 1	F10 Emetine 2	F11 Emetine 3	F12 Emetine 4
G1 Fluorouracil 1	G2 Fluorouracil 2	G3 Fluorouracil 3	G4 Fluorouracil 4	G5 Hydroxyurea 1	G6 Hydroxyurea 2	G7 Hydroxyurea 3	G8 Hydroxyurea 4	G9 Mechlorethamine 1	G10 Mechlorethamine 2	G11 Mechlorethamine 3	G12 Mechlorethamine 4
H1 Mercaptopurine 1	H2 Mercaptopurine 2	H3 Mercaptopurine 3	H4 Mercaptopurine 4	H5 Quinacrine Hydrochloride 1	H6 Quinacrine Hydrochloride 2	H7 Quinacrine Hydrochloride 3	H8 Quinacrine Hydrochloride 4	H9 Streptozosin 1	H10 Streptozosin 2	H11 Streptozosin 3	H12 Streptozosin 4

PM-M12 MicroPlate™ - Anti-Cancer Agents

A1 Negative Control	A2 Negative Control	A3 Negative Control	A4 Negative Control	A5 Tamoxifen Citrate 1	A6 Tamoxifen Citrate 2	A7 Tamoxifen Citrate 3	A8 Tamoxifen Citrate 4	A9 Thioguanine 1	A10 Thioguanine 2	A11 Thioguanine 3	A12 Thioguanine 4
B1 Acriflavinium Hydrochloride 1	B2 Acriflavinium Hydrochloride 2	B3 Acriflavinium Hydrochloride 3	B4 Acriflavinium Hydrochloride 4	B5 Pentamidine Isethionate 1	B6 Pentamidine Isethionate 2	B7 Pentamidine Isethionate 3	B8 Pentamidine Isethionate 4	B9 Mycophenolic Acid 1	B10 Mycophenolic Acid 2	B11 Mycophenolic Acid 3	B12 Mycophenolic Acid 4
C1 Aminopterin 1	C2 Aminopterin 2	C3 Aminopterin 3	C4 Aminopterin 4	C5 Berberine Chloride 1	C6 Berberine Chloride 2	C7 Berberine Chloride 3	C8 Berberine Chloride 4	C9 Emodin 1	C10 Emodin 2	C11 Emodin 3	C12 Emodin 4
D1 Puromycin Hydrochloride 1	D2 Puromycin Hydrochloride 2	D3 Puromycin Hydrochloride 3	D4 Puromycin Hydrochloride 4	D5 Neriifolin 1	D6 Neriifolin 2	D7 Neriifolin 3	D8 Neriifolin 4	D9 5-Fluoro-5'-Deoxyuridine 1	D10 5-Fluoro-5'-Deoxyuridine 2	D11 5-Fluoro-5'-Deoxyuridine 3	D12 5-Fluoro-5'-Deoxyuridine 4
E1 Carboplatin 1	E2 Carboplatin 2	E3 Carboplatin 3	E4 Carboplatin 4	E5 Cisplatin 1	E6 Cisplatin 2	E7 Cisplatin 3	E8 Cisplatin 4	E9 Zidovudine (AZT) 1	E10 Zidovudine (AZT) 2	E11 Zidovudine (AZT) 3	E12 Zidovudine (AZT) 4
F1 Azacytidine 1	F2 Azacytidine 2	F3 Azacytidine 3	F4 Azacytidine 4	F5 Cycloheximide 1	F6 Cycloheximide 2	F7 Cycloheximide 3	F8 Cycloheximide 4	F9 Azaserine 1	F10 Azaserine 2	F11 Azaserine 3	F12 Azaserine 4
G1 p-Fluoro-phenylalanine 1	G2 p-Fluoro-phenylalanine 2	G3 p-Fluoro-phenylalanine 3	G4 p-Fluoro-phenylalanine 4	G5 1,2-Dimethylhydrazine Hydrochloride 1	G6 1,2-Dimethylhydrazine Hydrochloride 2	G7 1,2-Dimethylhydrazine Hydrochloride 3	G8 1,2-Dimethylhydrazine Hydrochloride 4	G9 Phenethyl caffeate (CAPE) 1	G10 Phenethyl caffeate (CAPE) 2	G11 Phenethyl caffeate (CAPE) 3	G12 Phenethyl caffeate (CAPE) 4
H1 Camptothecin 1	H2 Camptothecin 2	H3 Camptothecin 3	H4 Camptothecin 4	H5 Amygdalin 1	H6 Amygdalin 2	H7 Amygdalin 3	H8 Amygdalin 4	H9 Ellagic Acid 1	H10 Ellagic Acid 2	H11 Ellagic Acid 3	H12 Ellagic Acid 4

PM-M13 MicroPlate™ - Anti-Cancer Agents

A1 Negative Control	A2 Negative Control	A3 Negative Control	A4 Negative Control	A5 Monocrotaline 1	A6 Monocrotaline 2	A7 Monocrotaline 3	A8 Monocrotaline 4	A9 Altretamine 1	A10 Altretamine 2	A11 Altretamine 3	A12 Altretamine 4
B1 Carmustine 1	B2 Carmustine 2	B3 Carmustine 3	B4 Carmustine 4	B5 Mitoxantrone Hydrochloride 1	B6 Mitoxantrone Hydrochloride 2	B7 Mitoxantrone Hydrochloride 3	B8 Mitoxantrone Hydrochloride 4	B9 Urethane 1	B10 Urethane 2	B11 Urethane 3	B12 Urethane 4
C1 Thiotepa 1	C2 Thiotepa 2	C3 Thiotepa 3	C4 Thiotepa 4	C5 Thiodiglycol 1	C6 Thiodiglycol 2	C7 Thiodiglycol 3	C8 Thiodiglycol 4	C9 Pipobroman 1	C10 Pipobroman 2	C11 Pipobroman 3	C12 Pipobroman 4
D1 Etanidazole 1	D2 Etanidazole 2	D3 Etanidazole 3	D4 Etanidazole 4	D5 Semustine 1	D6 Semustine 2	D7 Semustine 3	D8 Semustine 4	D9 Gossypol 1	D10 Gossypol 2	D11 Gossypol 3	D12 Gossypol 4
E1 Formestane 1	E2 Formestane 2	E3 Formestane 3	E4 Formestane 4	E5 Ancitabine Hydrochloride 1	E6 Ancitabine Hydrochloride 2	E7 Ancitabine Hydrochloride 3	E8 Ancitabine Hydrochloride 4	E9 Nimustine 1	E10 Nimustine 2	E11 Nimustine 3	E12 Nimustine 4
F1 Aminolevulinic Acid Hydrochloride 1	F2 Aminolevulinic Acid Hydrochloride 2	F3 Aminolevulinic Acid Hydrochloride 3	F4 Aminolevulinic Acid Hydrochloride 4	F5 Picropodo-phyllotoxin 1	F6 Picropodo-phyllotoxin 2	F7 Picropodo-phyllotoxin 3	F8 Picropodo-phyllotoxin 4	F9 beta-Peltatin 1	F10 beta-Peltatin 2	F11 beta-Peltatin 3	F12 beta-Peltatin 4
G1 Perillyl Alcohol 1	G2 Perillyl Alcohol 2	G3 Perillyl Alcohol 3	G4 Perillyl Alcohol 4	G5 Dibenzoylmethane 1	G6 Dibenzoylmethane 2	G7 Dibenzoylmethane 3	G8 Dibenzoylmethane 4	G9 6-Amino nicotinamide 1	G10 6-Amino nicotinamide 2	G11 6-Amino nicotinamide 3	G12 6-Amino nicotinamide 4
H1 Carmofur 1	H2 Carmofur 2	H3 Carmofur 3	H4 Carmofur 4	H5 Indole-3-Carbinol 1	H6 Indole-3-Carbinol 2	H7 Indole-3-Carbinol 3	H8 Indole-3-Carbinol 4	H9 Rifaximin 1	H10 Rifaximin 2	H11 Rifaximin 3	H12 Rifaximin 4

PM-M14 MicroPlate™ - Anti-Cancer Agents

A1 Negative Control	A2 Negative Control	A3 Negative Control	A4 Negative Control	A5 Cepharanthine 1	A6 Cepharanthine 2	A7 Cepharanthine 3	A8 Cepharanthine 4	A9 4'-Demethyl epipodophyllotoxin 1	A10 4'-Demethyl epipodophyllotoxin 2	A11 4'-Demethyl epipodophyllotoxin 3	A12 4'-Demethyl epipodophyllotoxin 4
B1 Miltefosine 1	B2 Miltefosine 2	B3 Miltefosine 3	B4 Miltefosine 4	B5 Elaidyl phosphocholine 1	B6 Elaidyl phosphocholine 2	B7 Elaidyl phosphocholine 3	B8 Elaidyl phosphocholine 4	B9 Podofilox 1	B10 Podofilox 2	B11 Podofilox 3	B12 Podofilox 4
C1 Colchicine 1	C2 Colchicine 2	C3 Colchicine 3	C4 Colchicine 4	C5 Methotrexate 1	C6 Methotrexate 2	C7 Methotrexate 3	C8 Methotrexate 4	C9 Acivicin 1	C10 Acivicin 2	C11 Acivicin 3	C12 Acivicin 4
D1 Floxuridine 1	D2 Floxuridine 2	D3 Floxuridine 3	D4 Floxuridine 4	D5 Lefunomide 1	D6 Lefunomide 2	D7 Lefunomide 3	D8 Lefunomide 4	D9 Rapamycin 1	D10 Rapamycin 2	D11 Rapamycin 3	D12 Rapamycin 4
E1 13-cis Retinoic Acid 1	E2 13-cis Retinoic Acid 2	E3 13-cis Retinoic Acid 3	E4 13-cis Retinoic Acid 4	E5 All-trans Retinoic Acid 1	E6 All-trans Retinoic Acid 2	E7 All-trans Retinoic Acid 3	E8 All-trans Retinoic Acid 4	E9 Piceatannol 1	E10 Piceatannol 2	E11 Piceatannol 3	E12 Piceatannol 4
F1 (+)-Catechin 1	F2 (+)-Catechin 2	F3 (+)-Catechin 3	F4 (+)-Catechin 4	F5 Mitomycin C 1	F6 Mitomycin C 2	F7 Mitomycin C 3	F8 Mitomycin C 4	F9 Cytosine-Beta-D-Arabinofuranoside 1	F10 Cytosine-Beta-D-Arabinofuranoside 2	F11 Cytosine-Beta-D-Arabinofuranoside 3	F12 Cytosine-Beta-D-Arabinofuranoside 4
G1 Daunorubicin Hydrochloride 1	G2 Daunorubicin Hydrochloride 2	G3 Daunorubicin Hydrochloride 3	G4 Daunorubicin Hydrochloride 4	G5 Doxorubicin Hydrochloride 1	G6 Doxorubicin Hydrochloride 2	G7 Doxorubicin Hydrochloride 3	G8 Doxorubicin Hydrochloride 4	G9 Etoposide 1	G10 Etoposide 2	G11 Etoposide 3	G12 Etoposide 4
H1 Nocodazole 1	H2 Nocodazole 2	H3 Nocodazole 3	H4 Nocodazole 4	H5 Quercetin Dihydrate 1	H6 Quercetin Dihydrate 2	H7 Quercetin Dihydrate 3	H8 Quercetin Dihydrate 4	H9 Vinblastine Sulfate 1	H10 Vinblastine Sulfate 2	H11 Vinblastine Sulfate 3	H12 Vinblastine Sulfate 4

V. Protocols, PM-M5 to M14

a. PM-M5 to M8 Protocols

These PMs are designed to facilitate study of the effects ions, hormones, and other metabolic effectors have on the metabolism, growth rate, or productivity of various cell lines under a variety of assay conditions. For example, one could prepare a suspension of cells in a culture medium containing either D-glucose or alternative carbon sources from PM-M1 to M4 that a cell can metabolize (e.g., D-fructose, L-lactic acid, L-alanine), and then, by dispensing this cell suspension into PM-M5 to M8, test how metabolism, growth, or productivity is affected by these agents.

The standard protocol outlined below in section c. would be followed with additional details and guidance provided on pages 7-10. Most commonly, these assays would be run in a serum-free medium since serum binding could complicate interpretation of results. In such cases, we typically find that, at a minimum the concentration of L-glutamine must be at least 2mM to replace the nitrogen nutrition provided by serum. Other hormones and growth factors are sometimes also required in serum free media.

b. PM-M11 to M14 Protocols

These PMs are designed to facilitate study of the sensitivity of cells to a diverse set of anti-cancer agents that can kill cells by a variety of modes of action. The anti-cancer agents may also alter the metabolism, growth rate, or productivity of cells. For example, one could test the sensitivity of any cell line to this set of 92 cytotoxic drugs with cells metabolizing different energy sources. Cancer cells typically exhibit the Warburg effect with increased dependence on glucose and altered energy metabolism. One could prepare a suspension of cells in a culture medium containing either D-glucose or alternative carbon sources from PM-M1 to M4 that a cell can metabolize (e.g., D-fructose, L-lactic acid, L-alanine) and then, by dispensing this cell suspension into PM-M11 to M14, test how toxicity, growth, or productivity is modulated in the presence of the various anti-cancer agents.

The standard protocol outlined below in section c. would be followed with additional details and guidance provided on pages 7-10. These PMs are also designed to be used in comparative assays of genetically related cell lines to examine how genetic changes, such as activation of oncogenes or multi-drug resistance pumps alters the susceptibility to a wide range of chemical agents. Most commonly, media like RPMI-1640 or DMEM would be used with these plates. In addition, these assays may be run in a serum-free medium since serum binding can complicate interpretation of results. In such cases, we typically find that, at a minimum the concentration of L-glutamine must be at least 2mM to replace the nitrogen nutrition provided by serum. Other hormones and growth factors are sometimes also required in serum free media.

c. Standard Protocol

The standard testing protocol has 4 simple steps:

1. Prepare a cell suspension at 4 x10^5 (PM-M5 to M8) or 4 x10^4 (PM-M11 to M14) cells/ml in an appropriate inoculation medium
2. Dispense 50 µl of the cell suspension into the wells of the PM panels and incubate the PM panels for 40 hours at 37° C. in an appropriate atmosphere
3. Dispense 10 µl of Redox Dye Mix into the wells, seal the plate with tape and incubate until sufficient dye reduction and color formation is observed
4. Measure the reduced dye (formazan) spectrophotometrically

VI. General Considerations

a. Light Sensitivity

As with other tetrazolium compounds, Biolog Redox Dye Mixes are light sensitive and supplied in amber containers. Discoloration may occur if solutions are stored improperly. This discoloration can cause higher background A_{590} values, making it difficult to quantify the cell-mediated tetrazolium reduction. Dye Mixes that become discolored and have unacceptably high A_{590} background values, should be discarded.

b. Chemical Safety and Stability

Material Data Safety sheets for these products are available from Biolog. The toxicological properties of the Dye Mixes have not been thoroughly investigated, so caution should be used in handling them. Tetrazolium compounds are generally classified as irritants. Suitable precautions should be taken also in the disposal of this product.

The wells of the PM panels are coated by dispensing chemicals in appropriate solvents and then drying them in the wells. It is expected that most if not all chemicals will remain stable and active over the shelf life of the products. However, because of the large number and diverse nature of the chemicals dried in the wells, Biolog cannot guarantee the stability and full activity of all chemicals.

c. Background Absorbance

A slight amount of 590 nm absorbance occurs due to abiotic reduction in serum-containing culture medium incubated with either Dye Mix solution. The type and pH of the culture medium, type and concentration of serum, temperature, length of exposure to light and any chemicals added to the culture medium may contribute to formazan production and consequent increase in A_{590} background values.

For example, culture medium at elevated pH or extended exposure to direct light may cause an accelerated non-cell mediated reduction of tetrazolium salts. Additionally, reducing substances including ascorbic acid and NADH, or sulfhydryl-containing compounds, such as L-cysteine, glutathione, coenzyme A, and dithiothreitol, and even strongly reducing sugars such as ribose and xylose can reduce tetrazolium salts nonenzymatically. Such chemicals may be ingredients of the culture medium or added to the cultures to alter assay conditions.

Background A_{590} values for Biolog Redox Dye Mix MA and MB are low. In RPMI medium lacking phenol red but containing 5% serum and Pen/Strep, Redox Dye Mix MA produced an A_{590} value of 0.007 ± 0.001 after 1 hr at $37^{o}C$. For Redox Dye Mix MB, the A_{590} value was 0.023 ± 0.002 units after 5 hours at $37^{o}C$. Unexpectedly high A_{590} values may indicate chemical interference from test compounds. This can be confirmed by measuring A_{590} values from control wells containing medium without cells at various concentrations of test compound.

If background A_{590} is significant using your experimental conditions, correct for it as follows. Prepare a triplicate set of control wells (without cells) containing the same volumes of culture medium and dye mix solution as in the experimental wells. Subtract the average A_{590} from these "no cell" control wells from all other A_{590} values to yield corrected absorbance values.

d. Optional Wavelengths to Read Absorbance

The reduced form of the Biolog Redox Dye Mixes absorbs maximally at 590 nm (Fig. 4) and this wavelength is recommended for determining the amount of formazan produced. If the microplate reader available does not have a 590 nm filter, wavelengths near 590 nm may be employed but this will decrease the assay sensitivity. Moreover, interference from colored chemicals added when performing chemosensitivity experiments may also result. Some chemicals that may be interesting to add to assays are yellow, orange, or brown with high absorbance between 400 nm and 500 nm.

e. Dual Wavelength Reading

It is preferable to collect data in dual wavelength mode with a second reference wavelength at 750 nm. Use of this second reference wavelength eliminates background absorbance contributed by cell debris, fingerprints, etc. The most accurate and sensitive readings are obtained by using $A_{590-750}$ values.

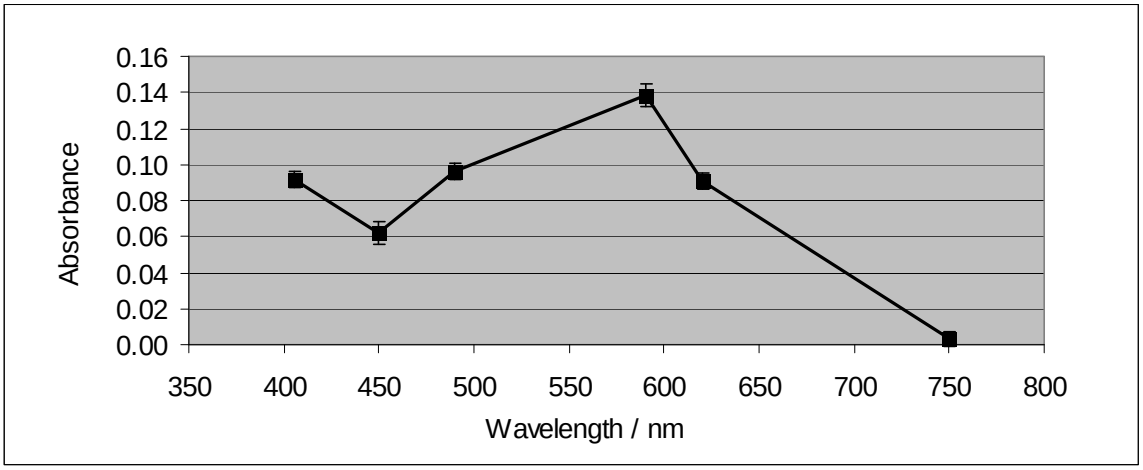

Figure 4. Absorption properties of reduced purple formazan generated by cells using either Biolog Redox Dye Mixes MA or MB. Maximal absorbance is at 590 nm and no absorbance at 750 nm is detectable. Error bars represent one standard deviation from 4 independent readings (n= 4).

f. Blood Cell Assays

Blood cells produce less formazan than other cell types and typically they should be assayed using Biolog Redox Dye Mix MB. However, individual cell lines may vary so both dye mixes should be tested before selecting one.

g. Cell Number Optimization

The number of cells per well in PM experiments and the incubation time can be altered, but such changes may produce suboptimal assay results. Increasing cell number will increase the rate of dye reduction, but such increases may lead to depletion of the substrate in the well before the color assay is performed. The time that cells incubate before dye is added can also be reduced, but this may lead to higher levels of dye reduction in the "no substrate" well and decreased signal to background ratios. The recommended protocol using 20,000 cells per well and adding Biolog Redox Dye Mixes after approximately 40 hr of incubation at 37°C works well for all cell lines tested thus far.

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