BIOLOG

User Defined Substrate Utilization Test Panel

MT2 MicroPlate™

A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12

FIGURE 1. Layout of MT2 MicroPlate™

INTRODUCTION

A 96-well microplate designed to test the ability of the inoculated microorganism suspension to utilize (oxidize) a panel of different carbon sources. Each well of the panel contains a tetrazolium redox dye and a buffered nutrient medium that has been developed and optimized for a wide variety of bacteria. Unlike other Biolog MicroPlates™ however, the carbon sources have been omitted so the wells in this regard are "empty" (MT). For biodegradation studies, this provides the user with flexibility in selecting an array of appropriate carbon sources. At the user's discretion, carbon sources may be added either before or after inoculating with a cell suspension. Volatile and hydrophobic carbon sources can also be used in this panel. Ideally, about 0.3 mg of carbon source (e.g. 15µl of a 2% stock solution) should be added to each well, however utilization of much lower levels (e.g. 20-200 ppm) can often be detected.

MT2 MICROPLATE ™

The MT2 MicroPlate™ (Biolog Cat. # 1013) provides a standardized micromethod for performing up to 95 carbon source utilization tests in a single panel. The user has complete flexibility in selecting the carbon sources and in configuring the tests within the panel (for example by row or by column). Biolog's MicroLog™ 2 or MicroLog™ 3 software may be used to construct a customized database to identify or compare specific strains based on their metabolic patterns in user-defined MT2 MicroPlates™.

Examples of potential uses are:
(1) **For bioremediation studies**, either pure cultures or mixed cultures such as direct environmental specimens can be easily tested against a set of xenobiotic chemicals to see which chemicals can be metabolized and to compare their rates of utilization.

(2) **For metabolic research,** a single bacterium can be tested against any set of carbon sources, or a single carbon source can be tested against any set of bacteria of interest.

DESCRIPTION

Biolog MicroPlates™ test the ability of a microorganism to utilize or oxidize compounds from a preselected panel of carbon sources. The test yields a characteristic pattern of purple wells which constitutes a **"Metabolic Fingerprint"** of the test organism(s).[1,2,3]

The wells of the MT2 MicroPlate™ are "empty" (MT) in that they do not contain any carbon sources. The user can load any set of carbon sources or carbon source mixtures into the wells of the panel. Each well already contains the buffered nutrient medium and the tetrazolium chemistry found in the Biolog GN2 MicroPlate™. Tetrazolium violet is used as a redox dye to colorimetrically indicate utilization of the carbon sources.

Ideally, about 0.3 mg of the carbon source (e.g. 15µl of a 2% stock solution) should be added to each well; however utilization of much lower levels (e.g. 20 to 200 ppm) may be detectable.[4] The carbon source can be pipetted into the well either before or after introduction of the cell suspension. Soluble carbon sources can be added either as a liquid or as a liquid which is dried. Insoluble hydrophobic chemicals can be added in solid crystalline form. A modified protocol has also been devised for adding volatile chemicals in the gaseous phase.[5] For each cell suspension tested, it is important to include a negative control well (with no carbon source) as a reference well for each test set.

Testing is performed as follows. Bacterial cells are suspended in sterile water, saline, or GN-GP IF (Biolog Cat. # 72101). Then the cell suspension is inoculated into the MT2 MicroPlate™, 150µl per well. The density of the cell suspension is an important variable that should be assessed and optimized. All of the wells start our colorless when inoculated. In wells that contain a chemical that is oxidized, there is a burst of respiration and the cells reduce the tetrazolium dye forming a purple color. Other wells remain colorless, as does the reference well with no carbon.

The MicroPlates™ are incubated for an appropriate time (usually 4 – 48 hours) to allow the pattern to form. The pattern of purple wells can then be keyed into Biolog's MicroLog™ 2 or the MicroLog™ 3 computer software which can cross-reference the pattern to a user-generated library of species or strains.

PRECAUTIONS

To obtain accurate and reproducible results, be sure to carefully follow the recommendations below.

- **Sterile** components and sterile techniques must be used in set-up procedures. Contamination will affect results.
- **Disposable** glassware or plasticware should be used to handle all cell suspensions and solutions. Glassware that has been washed may contain trace amounts of soap or detergent that can adversely affect results with sensitive strains.
- **Calibrate** your turbidimeter carefully and always prepare your inoculum within the specified density range.
- **Always keep in mind** that you are testing the metabolic properties of **live cells**. Some strains can lose their metabolic vigor when subjected to stresses (e.g. temperature, pH, osmolarity) for even a few minutes. To get the best performance possible from these MicroPlates™, be aware that the cells are alive and be conscious of and careful with how you handle them.

REFERENCES

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