

**EcoPlate™**

A1 Water	A2 β-Methyl-D-Glucoside	A3 D-Galactonic Acid γ-Lactone	A4 L-Arginine	A1 Water	A2 β-Methyl-D-Glucoside	A3 D-Galactonic Acid γ-Lactone	A4 L-Arginine	A1 Water	A2 β-Methyl-D-Glucoside	A3 D-Galactonic Acid γ-Lactone	A4 L-Arginine
B1 Pyruvic Acid Methyl Ester	B2 D-Xylose	B3 D-Galacturonic Acid	B4 L-Asparagine	B1 Pyruvic Acid Methyl Ester	B2 D-Xylose	B3 D-Galacturonic Acid	B4 L-Asparagine	B1 Pyruvic Acid Methyl Ester	B2 D-Xylose	B3 D-Galacturonic Acid	B4 L-Asparagine
C1 Tween 40	C2 i-Erythritol	C3 2-Hydroxy Benzoic Acid	C4 L-Phenylalanine	C1 Tween 40	C2 i-Erythritol	C3 2-Hydroxy Benzoic Acid	C4 L-Phenylalanine	C1 Tween 40	C2 i-Erythritol	C3 2-Hydroxy Benzoic Acid	C4 L-Phenylalanine
D1 Tween 80	D2 D-Mannitol	D3 4-Hydroxy Benzoic Acid	D4 L-Serine	D1 Tween 80	D2 D-Mannitol	D3 4-Hydroxy Benzoic Acid	D4 L-Serine	D1 Tween 80	D2 D-Mannitol	D3 4-Hydroxy Benzoic Acid	D4 L-Serine
E1 α-Cyclodextrin	E2 N-Acetyl-D-Glucosamine	E3 γ-Hydroxybutyric Acid	E4 L-Threonine	E1 α-Cyclodextrin	E2 N-Acetyl-D-Glucosamine	E3 γ-Hydroxybutyric Acid	E4 L-Threonine	E1 α-Cyclodextrin	E2 N-Acetyl-D-Glucosamine	E3 γ-Hydroxybutyric Acid	E4 L-Threonine
F1 Glycogen	F2 D-Glucosaminic Acid	F3 Itaconic Acid	F4 Glycyl-L-Glutamic Acid	F1 Glycogen	F2 D-Glucosaminic Acid	F3 Itaconic Acid	F4 Glycyl-L-Glutamic Acid	F1 Glycogen	F2 D-Glucosaminic Acid	F3 Itaconic Acid	F4 Glycyl-L-Glutamic Acid
G1 D-Cellobiose	G2 Glucose-1-Phosphate	G3 α-Ketobutyric Acid	G4 Phenylethylamine	G1 D-Cellobiose	G2 Glucose-1-Phosphate	G3 α-Ketobutyric Acid	G4 Phenylethylamine	G1 D-Cellobiose	G2 Glucose-1-Phosphate	G3 α-Ketobutyric Acid	G4 Phenylethylamine
H1 α-D-Lactose	H2 D,L-α-Glycerol Phosphate	H3 D-Malic Acid	H4 Putrescine	H1 α-D-Lactose	H2 D,L-α-Glycerol Phosphate	H3 D-Malic Acid	H4 Putrescine	H1 α-D-Lactose	H2 D,L-α-Glycerol Phosphate	H3 D-Malic Acid	H4 Putrescine

FIGURE 1. Carbon Sources in EcoPlate

## INTRODUCTION

Microbial communities provide useful information about environmental change. Microorganisms are present in virtually all environments and are typically the first organisms to react to chemical and physical changes in the environment. Because they are near the bottom of the food chain, changes in microbial communities are often a precursor to changes in the health and viability of the environment as a whole.

The Biolog EcoPlate™ (Figure 1) was created specifically for community analysis and microbial ecological studies. It was originally designed at the request of a group of microbial ecologists that had been using the Biolog GN MicroPlate™, but wanted a panel that provided replicate sets of tests<sup>1</sup>.

Community analysis using the Biolog MicroPlates was originally described in 1991 by J. Garland and A. Mills<sup>2</sup>. Researchers found that by inoculating Biolog GN MicroPlates with a mixed culture of microorganisms and measuring the community fingerprint over time, they could ascertain characteristics about that community of microbes. This approach, called community-level physiological profiling, has been demonstrated to be effective at distinguishing spatial and

temporal changes in microbial communities. In applied ecological research, the MicroPlates are used as both an assay of the stability of a normal population and to detect and assess changes following the onset of an environmental variable.

Studies have been done in diverse applications of microbial ecology and have demonstrated the fundamental utility of Biolog MicroPlates in detecting population changes in soil, water, wastewater, activated sludge, compost, and industrial waste. The utility of the information has been documented in over 500 publications using Biolog technology to analyze microbial communities. A bibliography of publications is posted on the Biolog website at [www.biolog.com/mID\\_section\\_4.html](http://www.biolog.com/mID_section_4.html).

## ECOPLATE

The Biolog EcoPlate contains 31 of the most useful carbon sources for soil community analysis. These 31 carbon sources are repeated 3 times to give the scientist more replicates of the data. Communities of organisms will give a characteristic reaction pattern called a metabolic fingerprint. These fingerprint reaction patterns rapidly and easily provide a vast amount of information from a single Biolog MicroPlate.

The community reaction patterns are typically analyzed at defined time intervals over 2 to 5 days. The changes in the pattern are compared and analyzed using statistical analysis software. The most popular method of analysis of the data is via Principle Components Analysis (PCA) of average well color development (AWCD) data, but alternative methods may also offer advantages<sup>3-8</sup>. The changes observed in the fingerprint pattern provide useful data about the microbial population changes over time.

Biolog MicroPlates have been compared to other methods, such as phospholipid fatty-acid analysis, for monitoring community and ecological changes. The MicroPlates were found to be more sensitive to changes in the environment<sup>9</sup>. Biolog MicroPlates were also indicated as more sensitive to changes in major determinants such as temperature and water.

Similar analyses have been performed using the Biolog GN and GN2 MicroPlates. For certain applications the GN2 MicroPlates may be preferable to the EcoPlate. The individual application will dictate which MicroPlate is best suited for the community or ecological analysis.

## TYPICAL PROCEDURE<sup>3</sup>

**STEP 1:** Environmental samples are inoculated directly into Biolog MicroPlates either as aqueous samples or after suspension (soil, sludge, sediment, etc...).

**STEP 2:** The Biolog MicroPlates are incubated and analyzed at defined time intervals.

**STEP 3:** The community-level physiological profile is assessed for key characteristics:

- Pattern development (similarity)
- Rate of color change in each well
- Richness of well response (diversity)

Formation of purple color occurs when the microbes can utilize the carbon source and begin to respire. The respiration of the cells in the community reduces a tetrazolium dye that is included with the carbon source.

The reaction patterns are most effectively analyzed with a microplate reader, using the MicroStation System. However, any good microplate reader can be used to provide optical density (OD<sub>590</sub>) values.

Statistical analysis of the data is typically performed using standard software packages. Some researchers have found that PCA provides greater resolution than other methods of statistical analysis<sup>10</sup>.

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Ph: 03 9416 7177  
Fax: 03 8678 3956  
email: [info@cellbiosciences.com.au](mailto:info@cellbiosciences.com.au)  
Web: [www.cellbiosciences.com.au](http://www.cellbiosciences.com.au)

**BIOLOG**

21124 Cabot Blvd. Hayward, CA 94545

Telephone: 510-785-2564

Fax: 510-782-4639

[www.biolog.com](http://www.biolog.com)