MT2 MicroPlate™

Instructions for Use

(For *In Vitro* Diagnostic Use Only)

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Part# OOP 005, Rev A, JUL 2001

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

INSTRUCTIONS FOR USE OF THE BIOLOG MT2 MICROPLATE

Intended Use

The MT2MicroPlate™ test panel provides a standardized micromethod for performing up to 96 carbon source utilization tests in a single panel. The user has complete flexibility in selecting the carbon sources and configuring the tests within the panel (for example by row or by column). Biolog's MicroLog™ 2 or Biolog's MicroLog™ 3 computer software may be used to construct a customized data base to identify or compare specific strains based on their metabolic patterns in user defined MT2MicroPlates.

Examples of potential uses are: (1) **For bioremediation studies,** either pure cultures or direct enviromental specimens can be easily tested against a set of xenobiotic chemicals to see which chemicals can be utilized and to compare their rates of utilization. (2) **For metabolic research,** a single bacterium can be tested against any set of interesting carbon sources, or a single carbon source can be tested against any set of interesting bacteria.

Description

Biolog MicroPlates test the ability of a microorganism suspension to utilize or oxidize a preselected panel of carbon sources. The test yields a characteristic pattern of purple wells, which constitutes a **"Metabolic Fingerprint"** of the capacities of the inoculated organisms.[1, 2, 3]

The wells of the MT2MicroPlate are "empty" (MT) in that they do not contain any carbon sources. The user can load any set of 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 GN2MicroPlate. Tetrazolium violet is used as a redux dye in the panel to colormetrically 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. 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 been devised for adding volatile chemicals in the gaseous phase.[4] 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 prewarmed sterile saline, within the density range specified. Then the cell suspension is inoculated into the MicroPlate, 150µl per well. All of the wells start out 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 source.

The MicroPlates are incubated for an appropriate time (usually 4 hours or overnight) to allow the pattern to form. The pattern of purple wells can then be keyed into Biolog's MicroLog 2 or Biolog's 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 can 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.
- **Prewarm** the GN/GP IF and the MicroPlates to Room Temperature (RT) before use. Some species may be sensitive to cold shocks.
- **Calibrate** your turbidimeter carefully and always prepare your inoculum within the specified density range.
- **Culture media and number of times subcultured** prior to testing can be very important. Many strains will produce different metabolic patterns depending upon how they are grown prior to inoculation. Refer to the section titled "Specimen Preparation" for details.
- **Always keep in mind** that you are testing the metabolic properties of **live cells**. Some species can lose their metabolic vigor when subjected to stresses (e.g. temperature, pH, and osmolarity) for even a few seconds. 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.
- **Read** the entire product insert prior to using the MicroPlate.

On Receipt

Inspect each foil pouch and MicroPlate for damage in shipping. **Do not** use the panel if the foil has been punctured. Components of Biolog's chemistry are sensitive to temperature and light. To preserve the full shelf life, the MicroPlates must be **stored at 2-8 °C.** inside its foil pouch. The expiration date is printed on each pouch. **Do not** use the plate after the expiration date.

MATERIALS

Materials Provided

- 10 Biolog MT2MicroPlates (Biolog Catalog #1003). Each MicroPlate contains all the necessary nutrients and biochemicals required. The user may add any carbon sources of interest

Quality Control

Biolog test panels are tested and meet internal quality control standards before being released for sale. However, some laboratories may desire or may be required to perform independent validations on each manufacturing lot.

To test the performance of the MT2 MicroPlate use the 4 gram-negative strains specified below. These are available from Biolog as a set (Biolog Catalog #8001).

1. *Alcaligenes xylosoxydans* ATCC 27061
2. *Ochrobactrum anthropi* ATCC 49687
3. *Providencia stuartii* ATCC 33672
4. *Cedecea neteri* ATCC 33855

Inoculate each bacterium following the TEST PROCEDURES as specified. When lyophilized or frozen cultures are used, they should be subcultured at least twice before being tested.

Read the panels after overnight incubation. The resulting identification should correctly correspond to the identity of the quality control strain.

If the identification does not match, review the test procedures and check the purity of your culture. Repeat the test. Call Biolog Technical Service if you have further problems or questions.

Technical Assistance

For help or to **report problems** with this product contact Biolog Technical Service either by phone (510-785-2564) or by fax (510-782-4639) during business hours (7:30am to 5pm Pacific Standard Time), or contact your local Biolog Distribution Partner.

References

[1] Bochner, BR 1989. Sleuthing out Bacterial Identities. *Nature* 339:157-158.
[2] Bochner, BR 1989. "Breathprints" at the Microbial Level. *ASM News* 55:536-539.
[3] Garland, J. L. and A. L. Mills. 1991. Classification and Characterization of Heterotrophic Microbial Communities on the Basis of Patterns of Community-Level Sole-Carbon-Source Utilization. *Appl. and Environ. Microbiol.* 57:2351-2359
[4] Strong-Gunderson, J. M., A. V. Palumbo, and S. O. Scarborough. 1992. New Method for Rapidly Determining Utilization of Volatile Contaminants. Abstracts of the 92nd Meeting of the American Society for Microbiology 1992:371.

Trouble Shooting

If you experience a problem in using the GN2 MicroPlate, start by rereading these Instructions for Use and review whether you have deviated from the recommended procedures. Then refer to the list below.

If all wells are positive, make sure that:

- The strain is not a polysaccharide-producing capsulated bacterium. If it is, follow the procedure adding thioglycolate to the inoculating fluid described above for **"False Positive" color.**
- You are using a microorganism that is appropriate for the MT2 MicroPlate. Gram-positive species and oligotrophic gram-negative species may give all positive wells.
- You are not carrying over any nutrients into the inoculating fluid.
- Your inoculum density is not excessive – check the calibration of your turbidimeter.
- The A-1 well is not under-filled. It is used as a reference well by the MicroStation Reader.
- Your inoculum is not clumpy.

If all wells are negative, make sure that:

- You are using a microorganism that is appropriate for the MT2 MicroPlate. Gram-positive species and oligotrophic gram-negative species may give all negative wells.
- Your cells are freshly grown and you have used the recommended agar medium.
- The inoculating fluid was prewarmed, prepared correctly, has the correct pH and salinity, and does not contain preservatives.
- You are handling the cells with all disposable hardware (soap residues are toxic).
- Your inoculum density is sufficient – check the calibration of your turbidimeter.
- Your incubation temperature and atmosphere are correct for the organism that is being tested.
- The A-1 well is not over-filled. It is used as a reference well by the MicroStation Reader.

See the Microlog /Omnilog User Guide for further assistance in interpreting identification results.

Performance Characteristics

The MT2 MicroPlate performance characteristics have been determined by establishing a database from a large collection of clinical and environmental stock microorganisms. The database is designed to give identifications of all species in the database, in accordance with current standards of classical identification methods and current taxonomic nomenclature. **To obtain accurate and reproducible results, all procedures and recommendations in the product insert must be followed precisely.**

Limitations

The MT2 MicroPlate is designed to identify pure cultures of gram-negative bacteria. The panel will only recognize members of the species in the current database. Other gram-negative species will usually be reported out with the message "no identification." Atypical strains may also yield a similarity index that is less than 0.5 at 16-24 hours and therefore will be reported out as "no identification."

Materials Not Provided

- BUG Agar: BUG (Biolog Universal Growth) Agar dehydrated medium (Biolog Catalog #70101) or plated BUG Agar with 5% sheep blood (Biolog Catalog #71102).
- GN/GP-IF: Sterile disposable glass (borosilicate) test tubes, 20ml capacity (20 x 150 mm) containing 18 to 20 ml of sterile "gelling" inoculating fluid (0.40% NaCl, 0.03% Pluronic F-68, 0.02% Gellan Gum, Biolog Catalog #72101).
- Thioglycolate: Sterile sodium thioglycolate ampules (Biolog Catalog #73011)
- LongSwabs™ : Sterile 7-inch disposable cotton-tipped swabs (Biolog Catalog #3021-3023).
- Streakerz™ : Sterile 6-inch tapered wooden streaking sticks (Biolog Catalog #3025-3026).
- Sterile disposable 9 inch transfer pipets (Biolog Catalog #3019).
- Sterile disposable reservoirs for multichannel pipetters (Biolog Catalog #3002).
- 8-Channel Repeating Pipettor (Biolog Catalog #3501 or 3505)
- Sterile disposable pipettor tips (Biolog Catalog #3001).
- Turbidimeter (Biolog Catalog #3531 or 3532).
- Biolog Turbidity Standards: GN-ENT & AN (Biolog Catalog #3416), GN-NENT (Biolog Catalog #3411) and GP-COC & GP-ROD & GN-FAS (Biolog Catalog #3414).
- Incubator: 26°, 30° and 35-37° C.

Material Preparation

- **Fill** sterile disposable capped tubes with 18-20 ml of sterile, normal saline (0.85% NaCl). Prewarm to 28-35°C.
- **Remove** MicroPlates from refrigerator and warm to 28-35°C.
- **Add** the carbon sources of interest to the wells of the MicroPlate.

TEST PROCEDURES

Step 1. Culture Isolation on Biolog Recommended Media

- **Isolate a pure culture** on agar media.
- **Use Biolog recommended media** (BUG Agar with 5% sheep blood or Chocolate Agar) and incubate at 30° or 35-37° C +/- CO_2. All species that can be identified with the GN2 MicroPlate will grow under these conditions.

Step 2. Specimen Preparation and Characterization

- **Perform a Gram stain** on your isolate to verify that it is gram negative.
- **Observe the cell morphology** in the Gram stain: coccus or rod.
- **Characterize the bacterium** into one of the following groups: non-enteric (**GN-NENT**), enteric (**GN-ENT**), or fastidious (**GN-FAS**). This will determine the proper protocols for growing the bacterium (Step 2 below) and preparing the inoculum (Step 3 below).
- Follow the **GN-NENT protocol** if: (a) The bacterium grows at 30°, but poorly or not at all at 35-37°C. (b) The bacterium is oxidase positive. (c) The bacterium is oxidase negative and gives a K/K or K/A^{w} reaction on a TSI slant.
- Follow the **GN-ENT protocol** if the isolate grows well at 35-37°C, is oxidase negative, and gives an A/A or K/A reaction on a TSI slant.
- Follow the **GN-FAS protocol** if the bacterium requires Chocolate Agar or 6.5% CO_2 to grow, or grows very poorly on BUG+B, forming pinpoint colonies. Most GN-FAS bacteria (*Actinobacillus, Alysiella, Brucella, Capnocytophaga, CDC Group DF-3, CDC Group EF-4, Eikenella, Haemophilus , Kingella, Moraxella, Neisseria, Simonsiella, Suttonella, and Taylorella spp*.) are isolated from respiratory tracts of mammals.
- See table 5 for exceptions and details to the protocols.

- **Grow the bacterium using the recommended conditions.** The choice of **the agar medium is very important** since it must support growth and promote retention of full metabolic activity to accurately match the metabolic patterns in the GN database. See table on page 5 for details.
- **The cells must be freshly grown** since many strains lose viability and metabolic vigor in stationary phase. The recommended incubation period for most organisms is 4-24 hours.
- **If insufficient growth is obtained** to inoculate the panel, restreak heavily onto one or more agar plates. Incubate for 4-48 hours. This should give enough growth to inoculate the panel.

Step 3. Inoculum Preparation

- **Establish the acceptable turbidity range** on your turbidimeter. First, set the 100% transmittance adjustment using a clean, uninoculated GN/GP-IF tube. Then, determine the desired turbidity for each group of gram negatives with the appropriate turbidity standards (GN-NENT or GN-ENT & AN or GP-COC & GP-ROD & GN-FAS) described in the section titled "Materials." Using the Biolog turbidimeter and 20 mm diameter tubes, this should give transmittance levels of about 63% (GN-ENT & AN), 52% (GN-NENT), and 20% (GP-COC & GP-ROD & GN-FAS) respectively. These readings may vary slightly on different Biolog Turbidimeters. With other instruments or with other tubes, the transmittance readings may vary substantially.
- **Blank the turbidimeter** (transmittance = 100%) with a clean tube containing uninoculated GN/GP-IF. Because the tubes used are not optically uniform, they should be blanked individually and not rotated in the light path of the turbidimeter.
- **Add Sodium thioglycolate (5 mM)** to the GN/GP-IF for tubes that will be used **for GN-ENT and GN-FAS bacteria**. This can be done by **adding precisely 3 drops** from an ampule containing a concentrated solution of sodium thioglycolate. Thioglycolate is an anti-capsule agent[3,4] and partially or completely inhibits the purple color in the A-1 well and other negative wells that can form when bacteria "eat" their polysaccharide capsules as a carbon source. Thioglycolate also is a reducing agent that improves the reactions for "weak" strains such as the GN-FAS bacteria. **A few species of GN-NENT may also require thioglycolate** only if the A-1 well turns purple to the extent that the metabolic pattern is not readable. In this case, retest the strain adding thioglycolate as described. See the table on page 5 for more details.
- **Prepare a uniform suspension** as follows: Remove cells from the agar plate with a sterile swab so as not to carry over any nutrients from the agar medium into the suspension. Move the swab up and down the wall of the tube until the organism mixes with the fluid and becomes a confluent suspension. A sterile transfer pipet may also be used to mix the suspension without creating an aerosol. Check the suspension for clumps and if they are present, let the tube stand for several minutes and allow them to settle to the bottom. If the organism is difficult to suspend or highly pigmented, use the dry tube technique described in the User Guide for GP-ROD SB.
- **Adjust the inoculum density.** Watch as the meter needle goes toward the accept able turbidity range. The acceptable range is defined by the turbidity standard plus or minus 3% transmittance. **This must be done with precision since it estab lishes the oxygen concentration for the cells and for the redox chemistry.** The density can be lowered by adding more inoculating fluid or raised by adding more cells.
- **Inoculate the cell suspension** into the MicroPlate promptly. Some strains lose metabolic activity if held too long (more than 20-30 minutes) in inoculating fluid without nutrients.

Step 4. Inoculation of the MicroPlate

- **Label** the MicroPlate with the organism name/number.
- **Pour** the cell suspension into the multichannel pipet reservoir.
- **Fasten** 8 sterile tips securely onto the 8-Channel Repeating Pipettor. Refer to manufacturer's instructions.
- **Fill the tips** and check to see that all tips are filling equally. If not, refasten any loose tips.
- **Prime the tips** if you are using a manual pipettor by dispensing the first delivery back into the reservoir. The electronic pipettor performs priming automatically.
- **Fill all wells with precisely 150 µl.** Be careful not to carry over chemicals or splash from one well into another. Continue dispensing until the fluid level in the tips is low. Then refill the tips and dispense into the remaining wells.
- **The inoculating fluid will form a soft gel** shortly after the inoculum is added to the wells and the MicroPlate is incubated.
- **Cover the MicroPlate** with its lid.

Step 5. Incubation

- **Incubate the MicroPlate** using the same conditions that were found to be optimal for the bacterium in Steps 1 and 2 above.
- **Provide a source of moisture in your incubator** to help minimize dehydration of the outer wells of the MicroPlate. Placing the MicroPlates in a plastic container with wet paper towels on the bottom should be sufficient.
- **Incubate plates** for 4 to 6 hours and / or overnight (16-24 hours).

RESULTS

Reading and Interpretation of Results

- Read MicroPlates using Microlog 1, Microlog 2, Microlog 3 or Omnilog Software. Refer to the User Guide for instructions.
- Most species give dark, clearly discernible "positive" reactions. However, it is normal for the "positive" reactions of certain genera, especially some fastidious gram negatives, to be light or faint purple.
- **"False positive" color** is defined as purple color forming in the control well (A-1) and in other "negative" wells. This is typically caused by utilization by capsulated strains of their extracellular polysaccharide and is commonly observed with ***Klebsiella, Enterobacter,*** and ***Serratia*** strains, ***Salmonella typhimurium***, and a few GN-NENT strains, if sodium thioglycolate is not added to the inoculating fluid. If this occurs, the strain should be retested with the addition of sodium thioglycolate as described above in Test Procedures - Step 3. Some species may give a light purple "false positive" color. However, this faint color is not a problem so long as the "true positive" reactions are discernible.
- **It is a good general practice to take a 4-6 hour reading.** Many Gram-negative species give strong positive patterns after 4 to 6 hours and can be identified quickly. Some strains require overnight incubation to give an adequate pattern.
- For MicroPlates read **after 4-6 hours** of incubation, **the similarity index must be at least 0.75** and have good, strong positive reactions to be considered an acceptable species identification. **After 16-24 hours** of incubation, **the similarity index must be at least 0.50** to be considered acceptable. These two threshold values give comparable levels of accuracy.
- For any isolate that is identified as ***Salmonella* or *Shigella or E. coli O157:H7,*** we recommend confirmation by serology. ***Neisseria gonorrhoeae*** should also be con firmed. Appropriate caution and confirmation should be used for isolates suspected of being from the DP database.