GN2 MicroPlate™

A1 Water	**A2** α-Cyclodextrin	**A3** Dextrin	**A4** Glycogen	**A5** Tween 40	**A6** Tween 80	**A7** N-Acetyl-D-Galactosamine	**A8** N-Acetyl-D-Glucosamine	**A9** Adonitol	**A10** L-Arabinose	**A11** D-Arabitol	**A12** D-Cellobiose
B1 i-Erythritol	**B2** D-Fructose	**B3** L-Fucose	**B4** D-Galactose	**B5** Gentiobiose	**B6** α-D-Glucose	**B7** m-Inositol	**B8** α-D-Lactose	**B9** Lactulose	**B10** Maltose	**B11** D-Mannitol	**B12** D-Mannose
C1 D-Melibiose	**C2** β-Methyl-D-Glucoside	**C3** D-Psicose	**C4** D-Raffinose	**C5** L-Rhamnose	**C6** D-Sorbitol	**C7** Sucrose	**C8** D-Trehalose	**C9** Turanose	**C10** Xylitol	**C11** Pyruvic Acid Methyl Ester	**C12** Succinic Acid Mono-Methyl Ester
D1 Acetic Acid	**D2** Cis-Aconitic Acid	**D3** Citric Acid	**D4** Formic Acid	**D5** D-Galactonic Acid Lactone	**D6** D-Galacturonic Acid	**D7** D-Gluconic Acid	**D8** D-Glucosaminic Acid	**D9** D-Glucuronic Acid	**D10** α-Hydroxybutyric Acid	**D11** β-Hydroxybutyric Acid	**D12** γ-Hydroxybutyric Acid
E1 p-Hydroxy-phenlyacetic Acid	**E2** Itaconic Acid	**E3** α-Ketobutyric Acid	**E4** α-Ketoglutaric Acid	**E5** α-Ketovaleric Acid	**E6** D,L-Lactic Acid	**E7** Malonic Acid	**E8** Propionic Acid	**E9** Quinic Acid	**E10** D-Saccharic Acid	**E11** Sebacic Acid	**E12** Succinic Acid
F1 Bromosuccinic Acid	**F2** Succinamic Acid	**F3** Glucuronamide	**F4** L-Alaninamide	**F5** D-Alanine	**F6** L-Alanine	**F7** L-Alanyl-Glycine	**F8** L-Asparagine	**F9** L-Aspartic Acid	**F10** L-Glutamic Acid	**F11** Glycyl-L-Aspartic Acid	**F12** Glycyl-L-Glutamic Acid
G1 L-Histidine	**G2** Hydroxy-L-Proline	**G3** L-Leucine	**G4** L-Ornithine	**G5** L-Phenylalanine	**G6** L-Proline	**G7** L-Pyroglutamic Acid	**G8** D-Serine	**G9** L-Serine	**G10** L-Threonine	**G11** D,L-Carnitine	**G12** γ-Aminobutyric Acid
H1 Urocanic Acid	**H2** Inosine	**H3** Uridine	**H4** Thymidine	**H5** Phenylethyl-amine	**H6** Putrescine	**H7** 2-Aminoethanol	**H8** 2,3-Butanediol	**H9** Glycerol	**H10** D,L,α-Glycerol Phosphate	**H11** α-D-Glucose-1-Phosphate	**H12** D-Glucose-6-Phosphate

- **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 careful with how you handle them.
- **Read** the entire "Instructions for Use" prior to using the MicroPlate.

On Receipt

Inspect each foil pouch and MicroPlate for damage in shipping. To maintain the full shelf life, the MicroPlates must be **stored at 2-8° C** inside their foil pouch. **The expiration date** is printed on each pouch. Do not use the MicroPlates after the expiration date.

MATERIALS

Materials Provided

- 10 Biolog GN2 MicroPlates (Biolog Catalog #1011).

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); plated **Chocolate Agar** (Biolog Catalog #Bio-M1012).
- **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).
- **Transfer Pipets**: Sterile disposable 9 inch transfer pipets (Biolog Catalog #3019).
- **Reservoirs**: Sterile disposable reservoirs for multichannel pipettor (Biolog Catalog #3002).
- **Pipettor**: 8-Channel Repeating Pipettor (Biolog Catalog #3501 or 3505).
- **Pipettor Tips**: Sterile disposable pipettor tips (Biolog Catalog #3001).
- **Turbidimeter**: (Biolog Catalog #3531 or 3532).
- **Turbidity Standards**: GN-ENT (Biolog Catalog #3421), GN-NENT (Biolog Catalog #3411) and GP-COC & GP-ROD & GN-FAS (Biolog Catalog #3414).
- **Incubator**: 30° and 35-37°C.

Material Preparation

- **Prewarm** MicroPlates and tubes of GN/GP-IF to at least 25°C before use.

If all wells are negative, make sure that:

- You are using a microorganism that is appropriate for the GN2 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.
- Your incubation temperature and atmosphere are correct for the organism that is being tested.
- 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.
- The A-1 well is not over-filled. It is used as a reference well by the MicroStation and OmniLog.

See the MicroLog/OmniLog User Guide for further assistance in interpreting identification results.

Performance Characteristics

The GN2 MicroPlate performance characteristics have been determined by establishing a database from a large collection of clinical and environmental 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 these Instructions for Use must be followed precisely.

Limitations

The GN2 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."

Quality Control

Biolog MicroPlates 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 GN2 MicroPlate use the 4 gram-negative strains specified below. These are available from Biolog as a set (Biolog Catalog #8001).

1.	*Achromobacter xylosoxydans ss 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.

- **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[4,5] 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 (strong yellow/orange pigment) may also require thioglycolate.** See Table 1 for species 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. Start with isolated colonies and then go into areas of heavier growth if necessary. Twirl and press the swab against the inside surface of the tube **on the dry glass above the fluid line** to break up clumps and release cells. Move the swab up and down the wall of the tube until the organism mixes with the fluid and becomes a homogenous, clump-free suspension. A sterile transfer pipet may also be used to mix the suspension without creating an aerosol. If clumps are still present, let the tube stand for several minutes and allow them to settle to the bottom. If the organism is difficult to suspend (mucoid or dry), 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 acceptable turbidity range. The acceptable range is defined by the turbidity standard plus or minus 2% transmittance. **This must be done with precision since it establishes 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 (**no more than 20 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 inoculation.
- **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).

Table 1: Summary of Testing Procedures and Exceptions.

Microbe type	GN-NENT	GN-ENT	GN-FAS	EXCEPTIONS
Culture medium	BUG+B or TSA+B	BUG+B or TSA+B	CHOCOLATE	**GN-NENT:** *Vibrio* must be grown on BUG+B. Agricultural genera (e.g. *Acidovorax, Brenneria, Burkholderia, Comamonas, Delftia, Pectobacterium, Pseudomonas, Rhizobium, Xanthomonas*) may be grown on BUG without blood.
Temperature	30° C	35-37° C	35-37° C	**GN-NENT:** Use 35-37° C for cardio and respiratory species (e.g. *Bordetella, Cardiobacterium, Mannheimia, Oligella, Pasteurella, Riemerella, Rhizobium like-CF, and Psychrobacter phenylpyruvicus*). Use 26°C for *Janthinobacterium and Vibrio splendidus.* **GN-ENT:** Use 30° C for species from cool environments (e.g. *Edwardsiella ictaluri, Brenneria, Erwinia, Pectobacterium, Photorhabdus, Xenorhabdus*).
Atmosphere	Air	Air	6.5% CO2	
Inoculating fluid	GN/GP-IF	GN/GP-IF + thioglycolate	GN/GP-IF + thioglycolate	**GN-NENT:** Use thioglycolate with "false positive" strains (e.g. *Chryseobacterium, Flavobacterium, Sphingomonas, and Psychrobacter phenylpyruvicus*). **GN-ENT:** Do not use thioglycolate for *Brenneria, Erwinia, Photorhabdus*, and *Xenorhabdus.* **GN-FAS:** Do not use thioglycolate for *Francisella* and *Brucella.*
Inoculum turbidity	52% T	61% T	20% T	
Inoculum per well	150 µl	150 µl	150 µl	
Incubation time	4-6, 16-24 hours	4-6, 16-24 hours	4-6, 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.
- The color density in **each well is referenced against the negative control well**, A-1. All wells visually resembling the A-1 well should be scored as "negative" (-) and all wells with a noticeable purple color (compared to well A-1) should be scored as "positive" (+). Wells with extremely faint color, or with small purple flecks or clumps are best scored as "borderline" (\). Most species give dark, clearly discernible "positive" reactions. However, it is normal for the "positive" reactions of certain genera 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 at **4-6 hours** of incubation, **the similarity index must be at least 0.75** to be considered an acceptable species identification. At **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 confirmed. Appropriate caution and confirmation should be used for isolates suspected of being **Dangerous Pathogens** (i.e., from the DP database).

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 GN2 MicroPlate. Gram-positive species and oligotrophic gram-negative species may give all positive wells.
- You are not carrying over any nutrients from the agar growth medium into the inoculating fluid.
- Your inoculum is free of all clumps.
- 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 and OmniLog.

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 (refer to Table 1).

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 (below) and preparing the inoculum (Step 3 below).
- Follow the **GN-NENT protocol** if: (a) the bacterium is oxidase positive or (b) the bacterium is oxidase negative and gives a K/K or K/A^{w} reaction on a TSI slant. These bacteria usually grow best at 30°.
- Follow the **GN-ENT protocol** if the bacterium is oxidase negative, and gives an A/A or K/A reaction on a TSI slant. These bacteria usually grow best at 35-37°C.
- Follow the **GN-FAS protocol** if: (a) the bacterium requires Chocolate Agar or 6.5% CO_2 to grow, or (b) grows very poorly on BUG+B, forming pinpoint colonies. These bacteria usually grow best at 35-37°C. 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*) are isolated from the respiratory tract of mammals.
- **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.
- **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 (as a lawn) onto one or more agar plates. Incubate for 4-48 hours. This should give enough growth to inoculate the panel.
- **Exceptions to and details of these protocols are shown in Table 1**.

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 gram negative type with the appropriate turbidity standards (GN-NENT or GN-ENT 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 61% (GN-ENT), 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.

Technical Assistance

For help or to **report problems** with this product contact Biolog Technical Service either by phone (510-785-2564) by fax (510-782-4639) or by email (tech@biolog.com) during business hours (7:30 A.M. to 5 P.M. 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] Biolog, Inc., US Patent # 5,627,045.

[4] Franco-Buff, A, Domenico, P and Bochner, BR 1998. Inhibition of Capsule Production in Bacteria by Thioglycolate. *ASM Abstracts* 98:432.

[5] Biolog, Inc., US Patent # 6,046,020.

Note: Customers with MicroLog Software Release 4.01C or earlier may use product #3416 GN-ENT & AN Turbidity Standard in place of product #3421 GN-ENT Turbidity Standard and product #3427 AN Turbidity Standard.

INSTRUCTIONS FOR USE OF THE BIOLOG GN2 MICROPLATE

Intended Use

The GN2 MicroPlate™ test panel provides a standardized micromethod using 95 biochemical tests to identify / characterize a broad range of enteric, non-enteric, and fastidious gram-negative bacteria. Biolog's MicroLog™ 1, MicroLog 2, MicroLog 3, or OmniLog™ software is used to identify the bacterium from its metabolic pattern in the GN2 MicroPlate.

Description

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

All necessary nutrients and biochemicals are prefilled and dried into the 96 wells of the plate. Tetrazolium violet is used as a redox dye to colorimetrically indicate the utilization of the carbon sources.

Testing is performed very simply. The isolate to be identified is grown on agar medium and then suspended in a special "gelling" inoculating fluid[3] (GN/GP-IF) at the recommended cell density. Then the cell suspension is inoculated into the GN2 MicroPlate, 150µl per well. All of the wells start out colorless when inoculated. During incubation there is a burst of respiration in the wells that contain chemicals that can be oxidized and the cells reduce the tetrazolium dye forming a purple color. Negative wells remain colorless, as does the reference well (A-1) with no carbon source.

The MicroPlates are incubated for 4-6 hours and /or 16-24 hours to allow the pattern to form. The pattern of purple wells is then keyed into Biolog's MicroLog computer software, which automatically cross-references the pattern to an extensive library of species. If an adequate match is found, an identification of the isolate is made.

Precautions

To obtain accurate and reproducible results, **the recommendations below must be followed**.

- **Pure cultures** must be used to obtain identifications. The system is not designed to identify individual bacterial strains from within mixed cultures.
- **Culture media and repeated subculturing** prior to testing is very important. Many strains will produce different metabolic patterns depending upon how they are cultured prior to inoculation. Refer to the section titled "Specimen Preparation" and "Limitations" for details.
- **Sterile** components and asceptic techniques must be used in set-up procedures. Contamination will affect results.
- **Disposable** glassware should be used to handle all cell suspensions and solutions. Glassware that has been washed may contain trace amounts of soap or detergent that will affect results.
- **Prewarm** the GN/GP-IF and the MicroPlates to room temperature before use. Some species (e.g. *Neisseria*) are very sensitive to cold shocks.
- **Calibrate** your turbidimeter carefully and **always prepare your inoculum within the specified density range.** Refer to section titled "Inoculum Preparation".
- **Biolog's chemistry** contains components that are sensitive to temperature and light. Dark brown wells in the MicroPlate indicate deterioration of the carbon source. Some wells may have an inherent yellow or pink hue, which is normal.

GN2 MicroPlate™

Instructions for Use

(For *In Vitro* Diagnostic Use Only)

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Part# OOP 003,Rev A, OCT 2001