

GEN III MicroPlate™

Instructions for Use

Not for human in vitro diagnostic use



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INSTRUCTIONS FOR USE OF THE BIOLOG GEN III MICROPLATE™

Intended Use

The GEN III MicroPlate™ test panel provides a standardized micromethod using 94 biochemical tests to profile and identify a broad range of Gram-negative and Gram-positive bacteria^{1,2}. Biolog's Microbial Identification Systems software (e.g. OmniLog® Data Collection) is used to identify the bacterium from its phenotypic pattern in the GEN III MicroPlate.

Description

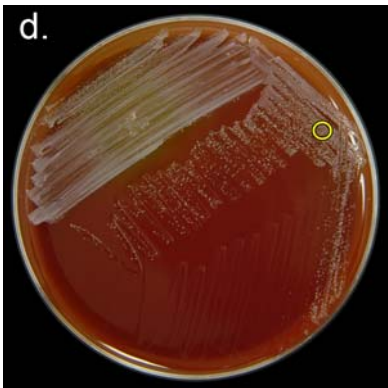
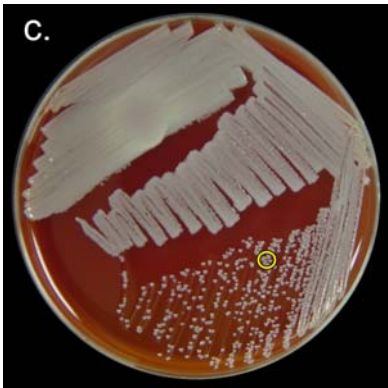
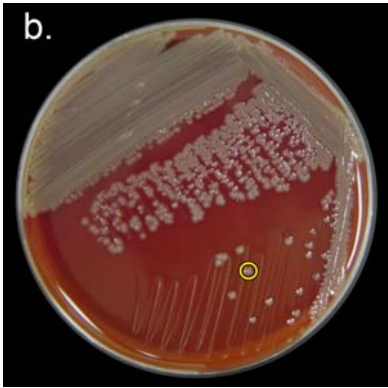
- The Biolog GEN III MicroPlate analyzes a microorganism in 94 phenotypic tests: 71 carbon source utilization assays (**Figure 1, columns 1-9**) and 23 chemical sensitivity assays (**Figure 1, columns 10-12**). The test panel provides a “**Phenotypic Fingerprint**” of the microorganism that can be used to identify it at the species level.
- All necessary nutrients and biochemicals are pre-filled and dried into the 96 wells of the MicroPlate. Tetrazolium redox dyes are used to colorimetrically indicate utilization of the carbon sources or resistance to inhibitory chemicals.
- Testing is performed very simply, as shown in **Figure 2**. The isolate to be identified is grown on agar medium and then suspended in a special “gelling” inoculating fluid³ (IF) at the recommended cell density. Then the cell suspension is inoculated into the GEN III MicroPlate, 100 µl per well, and the MicroPlate is incubated to allow the phenotypic fingerprint to form. All of the wells start out colorless when inoculated. During incubation there is increased respiration in the wells where cells can utilize a carbon source and/or grow. Increased respiration causes reduction of the tetrazolium redox dye, forming a purple color. Negative wells remain colorless, as does the negative control well (A-1) with no carbon source. There is also a positive control well (A-10) used as a reference for the chemical sensitivity assays in columns 10-12. After incubation, the phenotypic fingerprint of purple wells is compared to Biolog's extensive species library. If a match is found, a species level identification of the isolate is made.

Figure 1. Layout of assays in the MicroPlate

GEN III MicroPlate™

A1 Negative Control	A2 Dextrin	A3 D-Maltose	A4 D-Trehalose	A5 D-Cellobiose	A6 Gentiobiose	A7 Sucrose	A8 D-Turanose	A9 Stachyose	A10 Positive Control	A11 pH 6	A12 pH 5
B1 D-Raffinose	B2 α-D-Lactose	B3 D-Melibiose	B4 β-Methyl-D-Glucoside	B5 D-Salicin	B6 N-Acetyl-D-Glucosamine	B7 N-Acetyl-β-D-Mannosamine	B8 N-Acetyl-D-Galactosamine	B9 N-Acetyl Neuraminic Acid	B10 1% NaCl	B11 4% NaCl	B12 8% NaCl
C1 α-D-Glucose	C2 D-Mannose	C3 D-Fructose	C4 D-Galactose	C5 3-Methyl Glucose	C6 D-Fucose	C7 L-Fucose	C8 L-Rhamnose	C9 Inosine	C10 1% Sodium Lactate	C11 Fusidic Acid	C12 D-Serine
D1 D-Sorbitol	D2 D-Mannitol	D3 D-Arabitol	D4 myo-Inositol	D5 Glycerol	D6 D-Glucose-6-PO4	D7 D-Fructose-6-PO4	D8 D-Aspartic Acid	D9 D-Serine	D10 Troleandomycin	D11 Rifamycin SV	D12 Minocycline
E1 Gelatin	E2 Glycyl-L-Proline	E3 L-Alanine	E4 L-Arginine	E5 L-Aspartic Acid	E6 L-Glutamic Acid	E7 L-Histidine	E8 L-Pyroglutamic Acid	E9 L-Serine	E10 Lincomycin	E11 Guanidine HCl	E12 Niaproof 4
F1 Pectin	F2 D-Galacturonic Acid	F3 L-Galactonic Acid Lactone	F4 D-Gluconic Acid	F5 D-Glucuronic Acid	F6 Glucuronamide	F7 Mucic Acid	F8 Quinic Acid	F9 D-Saccharic Acid	F10 Vancomycin	F11 Tetrazolium Violet	F12 Tetrazolium Blue
G1 p-Hydroxy-Phenylacetic Acid	G2 Methyl Pyruvate	G3 D-Lactic Acid Methyl Ester	G4 L-Lactic Acid	G5 Citric Acid	G6 α-Keto-Glutaric Acid	G7 D-Malic Acid	G8 L-Malic Acid	G9 Bromo-Succinic Acid	G10 Nalidixic Acid	G11 Lithium Chloride	G12 Potassium Tellurite
H1 Tween 40	H2 γ-Amino-Butyric Acid	H3 α-Hydroxy-Butyric Acid	H4 β-Hydroxy-D,L-Butyric Acid	H5 α-Keto-Butyric Acid	H6 Acetoacetic Acid	H7 Propionic Acid	H8 Acetic Acid	H9 Formic Acid	H10 Aztreonam	H11 Sodium Butyrate	H12 Sodium Bromate

Figure 2. Steps in the testing protocol



Materials Provided

- **MicroPlates:** Catalog No.1030- Biolog GEN III MicroPlates (10/box).
- **On receipt, inspect** foil pouches and MicroPlates for damage in shipping. To maintain the full shelf life, the foil pouched MicroPlates must be **stored at 2-8° C**. The **expiration date** is printed on each pouch. Do not use the MicroPlates after the expiration date.

Materials Not Provided

- **Agar Culture Media:** Catalog No.71102-BUG Agar with 5% sheep blood (**BUG+B**); Catalog No. Bio-M1012- **Chocolate Agar**; Catalog No.70101- Biolog Dehydrated Growth Agar, 500 gm (**BUG Agar**).
- **Inoculating Fluid:** Catalog No.72401- **IF-A**, Catalog No.72402- **IF-B**, Catalog No. 72403- **IF-C**.
- **Inoculatorz™:** Catalog No.3321- Sterile disposable inoculator swabs (20x50); Catalog No.3323 (100x1).
- **Streakerz™:** Catalog No.3025- Sterile disposable wooden agar plate streakers (10x100); Catalog No.3026 (50x20).
- **Transfer Pipets:** Catalog No.3019- Sterile disposable 9 inch transfer pipets.
- **Reservoirs:** Catalog No.3102- Sterile disposable reservoirs.
- **Multichannel Pipettes:** Catalog No.3711- 8 channel electronic pipettor.
- **Pipet Tips:** Catalog No. 3201- Sterile racked pipet tips for Ovation multichannel pipettor; Catalog No. 3001- Matrix multichannel pipettor tips.
- **Turbidimeter:** Catalog No.3531- 110 volt model, Catalog No.3532 -220 volt model, Catalog No.3585 - 240 volt model.
- **Turbidity Standards:** Catalog No.3441 - 85% T; Catalog No.3440 - 65% T.

Determine Appropriate Protocol to Use (Inoculating Fluid and Cell Density)

- All protocols are performed in the same manner, the only difference being the choice of inoculating fluid (IF) and cell density for inoculation.
- **Protocol A** is used for the vast majority of species.
- **Protocol B** is used for a small number of strongly reducing species and capsulated species (primarily some strains of *Aeromonas*, *Vibrio*, and spore-forming Gram-positive rods). These species will give a false-positive result in the A-1 well with Protocol A. If this occurs, simply repeat the test using Protocol B.
- **Protocol C1** is used for slow growing bacteria that typically form pinpoint-sized colonies (less than 1 mm in diameter) on BUG+B Agar in 24 hours of growth (see example in **Figure 2.d.**). These are primarily microaerophilic and capnophilic Gram-positive cocci and tiny rods. See Table 1. below for a list.
- **Protocol C2** is used for fastidious, capnophilic, and very oxygen-sensitive bacteria that grow very slowly or not at all on BUG+B Agar. For example, it is used for fastidious Gram-negative species that would most likely be encountered from respiratory tract specimens after cultivation on Chocolate Agar with 6.5% CO₂. Some very oxygen-sensitive Gram-positive bacteria also require the higher inoculation density of Protocol C2. See Table 1. below for a list.
- If unsure of the appropriate test protocol, use protocol A. If the result fails to yield an identification because of a false-positive A-1 well, then use Protocol B. If the result fails because of insufficient positive carbon source reactions, then try, in succession, Protocols C1 and C2.

Table 1. Test Protocols

Protocol	IF	Cell Density	Species
A	A	90-98% T	Nearly all – this is the default protocol
B	B	90-98% T	Strongly reducing and capsule producing GN (e.g., some <i>Aeromonas</i> , <i>Vibrio</i>) and GP (e.g., some <i>Bacillus</i> , <i>Aneurinibacillus</i> , <i>Brevibacillus</i> , <i>Lysinibacillus</i> , <i>Paenibacillus</i> , and <i>Virgibacillus</i>)
C1	C	90-98% T	Microaerophilic, capnophilic GP (e.g., <i>Dolosicoccus</i> , <i>Dolosigranulum</i> , <i>Eremococcus</i> , <i>Gemella</i> , <i>Globicatella</i> , <i>Helcococcus</i> , <i>Ignavigranum</i> , <i>Lactobacillus</i> , <i>Lactococcus</i> , <i>Leuconostoc</i> , <i>Pediococcus</i> , <i>Streptococcus</i> , <i>Weissella</i> , and some <i>Aerococcus</i> , <i>Arcanobacterium</i> , <i>Corynebacterium</i> and <i>Enterococcus</i> sp.)
C2	C	62-68% T	Fastidious, capnophilic, oxygen sensitive GN (e.g., <i>Actinobacillus</i> , <i>Aggregatibacter</i> , <i>Alysiella</i> , <i>Avibacterium</i> , <i>Bergeriella</i> , <i>Bordetella</i> , <i>Capnocytophaga</i> , <i>Cardiobacterium</i> , <i>CDC Group DF-3</i> , <i>CDC Group EF-4</i> , <i>Conchiformibius</i> , <i>Dysgonomonas</i> , <i>Eikenella</i> , <i>Francisella</i> , <i>Gallibacterium</i> , <i>Gardnerella</i> , <i>Haemophilus</i> , <i>Histophilus</i> , <i>Kingella</i> , <i>Methylobacterium</i> , <i>Moraxella</i> , <i>Neisseria</i> , <i>Oligella</i> , <i>Ornithobacterium</i> , <i>Pasteurella</i> , <i>Simonsiella</i> , <i>Suttonella</i> , and <i>Taylorella</i>) and GP (<i>Actinomyces</i> , <i>Aerococcus</i> , <i>Alloiococcus</i> , <i>Arcanobacterium</i> , <i>Carnobacterium</i> , <i>Corynebacterium</i> , <i>Erysipelothrix</i> , <i>Granulicatella</i> , <i>Lactobacillus</i> , <i>Pediococcus</i> , and <i>Tetragenococcus</i>)

TEST PROCEDURE

Preparation

- Before starting, prewarm MicroPlates and IF to room temperature and review the entire protocol, including precautions.

Step 1. Culture Organism on Biolog Recommended Agar Media

- **Isolate a pure culture** on Biolog recommended agar media (**BUG+B** or **Chocolate Agar**) and incubate at 33° C. Some species may require special culture conditions, for example either lower or higher temperature (26° - 37° C.) and elevated CO₂ (6.5% - 10%).
- **Use of alternative media should be validated.** For laboratories that need to use agar media without blood, we recommend using **BUG Agar**. However, some species will grow extremely slowly or not at all if blood is omitted, for example the genera listed for Protocols C1 and C2 in Table 1. **R2A Agar** and Tryptic Soy Agar without or with blood (**TSA, TSA+B**) can be substituted, but they will not culture as wide a range of bacteria as BUG+B. Furthermore, their recipes and performance characteristics from different vendors may vary.
- **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. Spore-forming gram-positive bacteria (*Bacillus* and related genera) should be grown for less than 16 hours to help minimize sporulation.
- **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.

Step 2. Prepare Inoculum

- **Check the calibration of the turbidimeter** periodically. Use an appropriate turbidity standard (85% T or 65% T) and follow instructions in the turbidimeter manual to verify that the turbidimeter is calibrated and operating properly.
- **Blank the turbidimeter** with a clean tube (wiped clean of dirt and fingerprints) containing uninoculated IF. Because the tubes used are not optically uniform, they should be blanked individually. Set the 100% transmittance adjustment knob so that the meter reads 100%.
- **Prepare the inoculum at the desired turbidity.** The target **cell density should be in the range of 90-98%T for Protocols A, B, and C1. Protocol C2 requires a higher cell density of 62-68%T** for species that are sensitive to oxygen. **Use a cotton-tipped Inoculatorz swab** to pick up a 3 mm diameter area of cell growth from the surface of the agar plate. As shown in **Figure 2.a.**, grasp the swab at its tip and, holding the swab vertically, touch it to the cell growth. **Figure 2.b., c., and d.** show examples of fast, medium, and slow growing bacteria, and the yellow circle indicates where to touch the end of the cotton swab. For fast growing bacteria, touch a single colony, for medium growing bacteria, touch a cluster of colonies, and for slow growing bacteria touch the first area of confluent growth. Release the bacteria into the IF by rubbing the swab tip against the bottom of the tube containing IF as shown in **Figure 2.e.** Crush any cell clumps against the tube wall or remove them from the IF by catching them on the swab. Stir the IF with the swab to obtain a uniform cell suspension and read it in the turbidimeter, as shown in **Figure 2.f.** If the cell density is too low, add more cells. If the cell density is too high, add more IF.

For extremely clumpy bacteria that cannot be dispersed directly, use the following procedure. First prepare a dense suspension in 2 ml of IF as follows. **Use a sterile wooden Streakerz stick** to remove a clump of cell mass from the agar surface without gouging the agar. If the bacteria are extremely dry and embedded in the agar, use the edge of a **sterile glass microscope slide** to gently scrape a mass of cells onto the glass slide, again, without gouging the agar. The cells can then be scraped off the glass slide with a sterile Streakerz stick. Then use the Streakerz stick to deposit the cell mass onto the inner wall of a **dry tube**. Use the Streakerz stick to crush, break up, and spread the clumps of cells against and along the inner wall of the tube. Then add 2 ml of IF, and gradually slide the dispersed cells into the IF. The resulting cell suspension will be a mixture of suspended cells and residual clumps. Stand the tube in a rack for about 5 minutes and allow the clumps to settle to the bottom. Use a small pipet and transfer the suspended cells at the top into a fresh tube of IF to achieve the target cell density.

Step 3. Inoculate MicroPlate

- **Pour** the cell suspension into the multichannel pipet reservoir.
- **Fasten** 8 sterile tips securely onto the 8-Channel Repeating Pipettor and fill the tips by drawing up the cell suspension from the reservoir.
- **Fill all wells with precisely 100 µl** as shown in **Figure 2.g**. Be careful not to carry over chemicals or splash from one well into another. The inoculating fluid will form a soft gel shortly after inoculation.
- **Cover the MicroPlate** with its lid and eject the pipettor tips.

Step 4. Incubate MicroPlate

- **Place the MicroPlate** into the OmniLog incubator/reader as shown in **Figure 2.h.**, or into an incubator, for 3 to 36 hours. Incubate at 33° C., or use incubation conditions that were found to be optimal for the bacterium in Step 1.

RESULTS

Reading and Interpretation of Results

- **Read MicroPlates** using Biolog's Microbial Identification Systems software (e.g. OmniLog® Data Collection). Refer to the User Guide for instructions.
- **Biolog's Microbial Identification Systems Software performs all reading and interpretation of results.**
- The color densities in wells of the **carbon source utilization assays** in columns 1-9 are **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 (greater than 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.
- The color densities in wells of the **chemical sensitivity assays** in columns 10-12 are **referenced against the positive control well, A-10**. All wells showing significant sensitivity to the inhibitory chemical, with less than half the color of the A-10 well are considered "negative" (-) for growth. All other wells showing normal or near normal purple color (similar to well A-10) are considered "positive" (+). If there is uncertainty about the interpretation, it is best to score the well as "borderline" (∅).
- **"False positive" color** is defined as purple color forming in the negative control well (A-1) and in other "negative" wells. This is seen with only a few species such as from the genera *Aeromonas*, *Vibrio*, and *Bacillus*. If such a result occurs, the cells are simply retested with Protocol B and IF-B.
- **See Biolog's Microbial Identification Systems software User Guide for further assistance in interpreting identification results.**

Precautions

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

- **Read** the "Instructions for Use" prior to using the GEN III MicroPlate and follow the procedures.
- **Pure cultures must be used** to obtain identifications. The system is not designed to identify individual bacterial strains from within mixed cultures. The most common problem in identification is that microbiologists are not aware that they have a mixed culture. Streaking for isolated colonies may not be sufficient because isolated colonies can arise from a clump of cells as well as a single cell. Bacteria have sticky surfaces and they tightly adhere to other bacteria. **This is particularly a problem with mucoid bacteria, fresh environmental isolates, and staphylococci.** First, examine cultures with care using a dissecting microscope or some colony magnifying lens, to make sure that only one colony morphology is present in the culture. **If no species identification is obtained, you may still have a mixed culture.** Restreak the cells onto a multi-chromogenic agar medium and let the original agar plate and the chromogenic agar plate sit at room temperature for 3 or 4 days. Examine both plates carefully, looking for the outgrowth of "bumps" or non-uniform growth in the areas of confluent growth. On the chromogenic agar plate, look for more than one color. If necessary, reisolate the colony types that are present and perform the identification assay a second time.

- **Culture media and repeated subculturing** may affect the results. Strains may produce different phenotypic patterns depending upon how they are cultured prior to inoculation.
- **Sterile** components and aseptic 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 IF and the MicroPlates to room temperature before use. Some species (e.g., *Neisseria sp.*) are very sensitive to cold shocks.
- **Check the calibration of** your turbidimeter carefully and **always prepare your inoculum within the specified density range.**
- **Biolog's chemistry** contains components that are sensitive to temperature and light. Store the inoculating fluids in the dark with refrigeration. Brown or yellow wells in the GEN III MicroPlate indicate deterioration of the chemistry.
- **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 minutes. To get the best performance possible from these MicroPlates, be aware that the cells are alive and take care in how you handle them.

Trouble Shooting

If all wells in columns 1-9 are positive, make sure that:

- You are using a microorganism that is appropriate for the GEN III MicroPlate. If the bacterium is a strongly reducing or capsulated species causing false positive color in the A-1 well, repeat the test using Protocol B and IF-B.
- 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 Biolog's Microbial Identification Systems software.

If all wells in columns 1-9 are negative, make sure that:

- You are using a microorganism that is appropriate for the GEN III MicroPlate. Oligotrophic species or extremely slow growing or oxygen sensitive bacteria, for example, may give all negative wells.
- Your cells are freshly grown and you have used the recommended agar culture medium.
- Your incubation temperature and atmosphere are correct for the organism that is being tested.
- The inoculating fluid was stored correctly and was prewarmed prior to use.
- 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 Biolog's Microbial Identification Systems software.

Performance Characteristics

The GEN III MicroPlate performance characteristics have been determined by establishing a database using a large collection of microorganisms from diverse sources. 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 GEN III MicroPlate is designed to identify pure cultures of Gram-negative and Gram-positive bacteria. The panel will only identify members of the species in the current database. Other species will usually be reported out with the message "no identification." Atypical strains may also yield a low similarity index and therefore will be reported out as "no identification." **This product is not for human in vitro diagnostic use.** Some bacterial species are reportable to government and public health agencies in certain circumstances. For any isolate that is identified as *Salmonella* or *Shigella* or *E. coli O157:H7*, we recommend confirmation by serology. *Neisseria gonorrhoeae* identifications should also be confirmed. Appropriate caution and confirmation should be used for isolates suspected of being **Dangerous Pathogens**.

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 quality control checks on each manufacturing lot.

To test the performance of the GEN III MicroPlate use the 2 Gram-negative and 2 Gram-positive strains specified below using Protocol A. These are available from Biolog as a set (Biolog Catalog No.8050).

1. <i>Escherichia coli</i>	ATCC 11775
2. <i>Paenibacillus polymyxa</i>	ATCC 842
3. <i>Staphylococcus epidermidis</i>	ATCC 12228
4. <i>Stenotrophomonas maltophilia</i>	ATCC 13637

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

Read the panels after appropriate 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.

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 the Biolog Distribution Partner in your area.

General information, Certificates of Analysis and MSDS can now be found at www.biolog.com.

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.

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