YT MicroPlate™

Instructions for Use

(Not For Human *In Vitro* Diagnostic Use)

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YT MicroPlate™

A1 Water	**A2** Acetic Acid	**A3** Formic Acid	**A4** Propionic Acid	**A5** Succinic Acid	**A6** Succinic Acid Mono-Methyl Ester	**A7** L-Aspartic Acid	**A8** L-Glutamic Acid	**A9** L-Proline	**A10** D-Gluconic Acid	**A11** Dextrin	**A12** Inulin
B1 D-Cellobiose	**B2** Gentiobiose	**B3** Maltose	**B4** Maltotriose	**B5** D-Melezitose	**B6** D-Melibiose	**B7** Palatinose	**B8** D-Raffinose	**B9** Stachyose	**B10** Sucrose	**B11** D-Trehalose	**B12** Turanose
C1 N-Acetyl-D-Glucosamine	**C2** α-D-Glucose	**C3** D-Galactose	**C4** D-Psicose	**C5** L-Sorbose	**C6** Salicin	**C7** D-Mannitol	**C8** D-Sorbitol	**C9** D-Arabitol	**C10** Xylitol	**C11** Glycerol	**C12** Tween 80
D1 Water	**D2** Fumaric Acid	**D3** L-Malic Acid	**D4** Succinic Acid Mono-Methyl Ester	**D5** Bromosuccinic Acid	**D6** L-Glutamic Acid	**D7** γ-Aminobutyric Acid	**D8** α-Ketoglutaric Acid	**D9** 2 -Keto-D-Gluconic Acid	**D10** D-Gluconic Acid	**D11** Dextrin	**D12** Inulin
E1 D-Cellobiose	**E2** Gentiobiose	**E3** Maltose	**E4** Maltotriose	**E5** D-Melezitose	**E6** D-Melibiose	**E7** Palatinose	**E8** D-Raffinose	**E9** Stachyose	**E10** Sucrose	**E11** D-Trehalose	**E12** Turanose
F1 N-Acetyl-D – Glucosamine	**F2** D-Glucosamine	**F3** α-D-Glucose	**F4** D -Galactose	**F5** D-Psicose	**F6** L-Rhamnose	**F7** L-Sorbose	**F8** α-Methyl-D-Glucoside	**F9** β-Methyl-D-Glucoside	**F10** Amygdalin	**F11** Arbutin	**F12** Salicin
G1 Maltitol	**G2** D-Mannitol	**G3** D-Sorbitol	**G4** Adonitol	**G5** D-Arabitol	**G6** Xylitol	**G7** i-Erythritol	**G8** Glycerol	**G9** Tween 80	**G10** L-Arabinose	**G11** D-Arabinose	**G12** D-Ribose
H1 D-Xylose	**H2** Succinic Acid Mono-Methyl Ester plus D-Xylose	**H3** N-Acetyl-L-Glutamic Acid plus D-Xylose	**H4** Quinic Acid plus D-Xylose	**H5** D-Glucuronic Acid plus D-Xylose	**H6** Dextrin plus D-Xylose	**H7** α-D-Lactose plus D-Xylose	**H8** D-Melibiose plus D-Xylose	**H9** D-Galactose plus D-Xylose	**H10** m-Inositol plus D-Xylose	**H11** 1,2-Propanediol plus D-Xylose	**H12** Acetoin plus D-Xylose

Oxidation Tests Assimilation Tests

INSTRUCTIONS FOR USE OF THE BIOLOG YT MICROPLATE

Intended Use

The YT MicroPlate™ test panel provides a standardized micromethod using 94 biochemical tests to identify/characterize a broad range of yeasts. Biolog‘s MicroLog™ 3 software is used to identify the yeast from its metabolic pattern in the YT 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 and turbid 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 in some of the wells as a redox dye to colorimetrically indicate the oxidation of the carbon sources. Utilization of carbon sources in the other wells is indicated by an increase in turbidity.

Testing is performed very simply. The isolate to be identified is grown on agar medium and then suspended in YT Inoculating Fluid (P/N 72501) or prewarmed sterile water at the recommended cell density. Then the cell suspension is inoculated into the YT MicroPlate, 100µl per well. All of the wells start out colorless when inoculated. During incubation, there is an increase in respiration in wells that contain a chemical that can be utilized and the cells either reduce the tetrazolium dye forming a purple color or initiate growth leading to an increase in turbidity. There are two reference wells in the plate (A-1 and D-1) with no carbon source.

The MicroPlates are incubated for 24, 48, and/or 72 hours to allow the pattern to form. The metabolic pattern is then interpreted by Biolog‘s MicroLog 3 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 yeast 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 sections titled “Specimen Preparation” and “Limitations” for details.
- **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 YT Inoculating Fluid or water and the MicroPlates to room temperature before use.
- **Calibrate** 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. 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.

- **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. **Store** the MicroPlates 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. During shipment, the MicroPlates may be maintained at room temperature for a period of up to 12 days.

MATERIALS

Materials Provided

- 10 Biolog YT MicroPlates (Biolog Catalog #1005).

Materials Not Provided

- **BUY Agar**: BUY (Biolog Universal Yeast) Agar dehydrated medium (Biolog Catalog #70005) or plated BUY Agar (Biolog Catalog #71005).
- **YT Inoculating Fluid**: Prepared sterile disposable glass test tubes containing 12.5 mL of inoculating fluid (Biolog Catalog #72501).
- **Sterile water**: Sterile disposable glass (borosilicate) test tubes, 20ml capacity (20 x 150mm) containing 12 to 15 ml of sterile water, pH 5.5-7.0.
- **LongSwabs™** : Sterile 7-inch disposable cotton-tipped swabs (Biolog Catalog #3023).
- **Streakerz™** : Sterile 6-inch tapered wooden streaking sticks (Biolog Catalog #3026).
- **Transfer Pipets**: Sterile disposable 9-inch transfer pipets (Biolog Catalog #3019).
- **Reservoirs**: Sterile disposable reservoirs for multichannel pipettors (Biolog Catalog #3102).
- **Pipettor**: 8-Channel Repeating Pipettor (Biolog Catalog #3711 Ovation).
- **Pipettor Tips**: Sterile disposable pipettor tips (Biolog Catalog #3201 Ovation).
- **Turbidimeter**: (Biolog Catalog #3587).
- **Turbidity Standard**: YT (Biolog Catalog #3415).
- **Incubator**: 26-28°C.

Material Preparation

- **Prewarm** MicroPlates and tubes of YT Inoculating Fluid or sterile water to at least 25°C before use.

TEST PROCEDURES

Step 1. Culture Isolation on Biolog Recommended Media

- **Isolate a pure culture** on agar media.
- **Use Biolog recommended media** (BUY Agar) and incubate at 26-28°C. All species that can be identified with the YT MicroPlate will grow under these conditions.

Step 2. Specimen Preparation and Characterization

- **Perform a Wet prep or Gram stain** if necessary to verify that it is a yeast.
- **Grow the yeast 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 YT 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 24 to 48 hours.
- **If insufficient growth is obtained** to inoculate the panel, inoculate more than one agar plate. Incubate 24-48 hours.

Step 3. Inoculum Preparation

- **Establish the acceptable turbidity range** on your turbidimeter. First, set the 100% transmittance adjustment using a clean uninoculated water tube. Then, determine the desired turbidity with the YT Turbidity Standard described in the section titled “Materials“. Using the Biolog turbidimeter and 20 mm diameter tubes, this should give a transmittance level of about 47%. 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 water. Because the tubes used are not optically uniform, they should be blanked individually and not rotated in the light path of the turbidimeter.
- **Prepare a uniform suspension** as follows: Remove cells from the agar plate with a sterile cotton 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. The fluid then 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 present, let the tube stand for several minutes and allow them to settle to the bottom.
- **Adjust the inoculum density.** Watch that the meter needle goes toward the acceptable turbidity range. The acceptable turbidity range is defined as 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 water 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 minutes**) in water 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 100 µl**. Be careful not to carry over chemicals or splash from one well into another.
- **Cover the MicroPlate** with its lid.

Step 5. Incubation

- **Incubate the MicroPlate** at 26-28°C.
- **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 24, 48 or 72 hours, until a sufficient pattern is formed.

RESULTS

Reading and Interpretation of Results

- **Read MicroPlate using MicroLog 3** Software. Refer to the User Guide for instructions.
- **Interpretation of Results:** The color density or turbidity increase in each well is referenced against the negative control wells, A-1 and D-1. All wells optically resembling the negative control wells are scored as “negative” (-) and all wells with a noticeable increase in absorbance at 590 nm are scored as “positive” (+). Wells with an extremely slight increase in absorbance at 590 nm are scored as “borderline” (\). Typically, the patterns in the YT MicroPlate will be difficult or impossible to read and “score” by eye.
- **“False positive”** results are defined as purple color or turbidity forming in the control well (A-1 or D-1) and in other “negative” wells. Possible causes include utilization of extracellular polysaccharides, utilization of stored endogenous substrates, or utilization of lysed cell material. Improper use of the MicroPlate can also cause “false positive” color to occur. This is discussed in the section titled “Trouble Shooting”. Some yeast species metabolize carbon sources to form colored (e.g. brown) byproducts. These should be scored as “positive” reactions.
- For MicroPlates read at **24 hours** of incubation, **the similarity index must be at least 0.75** to be considered an acceptable species identification. At **48 or 72 hours** of incubation, **the similarity index must be at least 0.50** to be considered acceptable.

Trouble Shooting

If you experience a problem in using the YT 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:

- You are using a microorganism that is appropriate of the YT MicroPlate.
- You are not carrying over any nutrients from the agar growth medium into the inoculating fluid.
- Your inoculum density is not excessive. Re-check the calibration of your turbidimeter.
- Your inoculum is free of all clumps.
- The A-1 and D-1 wells are not under-filled. They are used as reference wells by the MicroStation™.

If all wells are negative, make sure that:

- You are using a microorganism that is appropriate for the YT MicroPlate.
- Your cells are freshly grown and you have used the recommended agar medium.
- The suspension fluid was prewarmed, has the correct pH, and does not contain preservatives.
- Your incubation temperature is correct for the organism that is being tested.
- You are handling the cells with all disposable hardware (soap residues are toxic).
- Your inoculum density is sufficient. Re-check the calibration of your turbidimeter.
- The A-1 and D-1 wells are not over-filled. They are used as reference wells by the MicroStation.

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

Performance Characteristics

The YT 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 these Instructions for Use must be followed precisely.

Limitations

The YT MicroPlate is designed to identify pure cultures of yeast. The panel will only recognize members of the species in the current database. Other yeast 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 72 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 YT MicroPlate use the 4 yeast strains specified below. These are available as a set from authorized distributors.

1.	Candida albicans	ATCC 10231
2.	Candida geochares	ATCC 36852
3.	Kluyveromyces marxianus (Candida kefyr)	ATCC 2512
4.	Galactomyces geotrichum (Geotrichum candidum)	ATCC 34614

Inoculate each yeast 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 48 hours of 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 any 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) by fax (510-782-4639) or by email (tech@biolog.com) during business hours (7:30A.M. to 5P.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.