YT Microplate Instructions For Use

For Research Use Only

Biolog, Inc.
21124 Cabot Blvd.
Hayward, CA 94545
www.biolog.com

Technical Assistance:
tech@biolog.com
510-785-2564

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Intended Use

The YT Microplate test panel provides a standardized method using 94 biochemical tests to identify and/or characterize a broad range of yeasts. Biolog's software is used to identify the yeast from its metabolic pattern in the YT Microplate. The YT Microplate is not for human in vitro diagnostic use. It is intended for research use only.

Description

Biolog Microplates test the ability of a microorganism to utilize or oxidize compounds from a preselected panel of phenotypic tests. The panel provides a phenotypic fingerprint of the microorganism, comprised of a characteristic pattern of purple and turbid wells, for making a species level identification.

All necessary nutrients and biochemicals are prefilled and dried into the 96 wells of each plate. Tetrazolium violet is used in some wells as a redox dye to colorimetrically indicate the ability of the organism to utilize the chemical in a specific well. Utilization of carbon sources in other wells is indicated by an increase in turbidity.

The isolate to be identified is grown on agar medium and later suspended in YT Inoculating Fluid, or prewarmed sterile water at the recommended cell density. Then the cell suspension is inoculated into the YT Microplate at 100 µl per well. Each well appears colorless when inoculated. During incubation, an increase in respiration occurs in wells with a chemical that can be utilized. 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 with no carbon source in the plate (A-1 and D-1).

The YT Microplates are incubated for 1 to 3 days, allowing the pattern to form. The metabolic pattern is then interpreted by Biolog's software, which automatically cross-references the pattern to an extensive library of species. If an adequate match is found, the isolate is identified.

Upon Receipt

1. Inspect each pouch containing the YT Microplates for damage from shipping. *DO NOT USE THE YT Microplates IF THE PACKAGING INDICATES SIGNS OF DAMAGE*.
2. Store the YT Microplates at 2-8° C inside their pouches to preserve full shelf life.
3. Ensure each YT Microplate is within the expiration date printed on each pouch. *DO NOT USE THE YT Microplates AFTER THE EXPIRATION DATE*.
4. During shipment, the YT Microplates may be maintained at room temperature for a period of up to 12 days.

Materials Required for Test Procedure

Materials Included

1. 10 Biolog YT Microplates (Biolog Catalog #1005)

Materials Not Included

1.	BUY Agar	BUY (Biolog Universal Yeast) Agar dehydrated medium (Biolog Catalog #70005) or plated BUY Agar (Biolog Catalog #71005).
2.	YT-Inoculating Fluid (YT-IF)	Prepared sterile disposable glass test tubes containing 12.5 ml of inoculating fluid (Biolog Catalog #72501).
3.	Sterile water	Sterile disposable glass (borosilicate) test tubes, 20ml capacity (20 x 150mm) containing 12 to 15 ml of sterile water.
4.	LongSwabs™	Sterile 7-inch disposable cotton-tipped swabs (Biolog Catalog #3023).
5.	Streakerz™	Sterile 6-inch tapered wooden streaking sticks (Biolog Catalog #3026).
6.	Transfer Pipets	Sterile disposable 9-inch transfer pipets (Biolog Catalog #3019).
7.	Reservoirs	Sterile disposable reservoirs for multichannel pipettors (Biolog Catalog #3102).
8.	Pipettor	8-Channel Repeating Pipettor (Biolog Catalog #3711).
9.	Pipettor Tips	Sterile disposable pipettor tips (Biolog Catalog #3201).
10.	Turbidimeter	Biolog Catalog #3587.

YT Microplate Map

A-1 Water	A-2 Acetic Acid	A-3 Formic Acid	A-4 Propionic Acid	A-5 Succinic Acid	A-6 Succinic Acid Mono-Methyl Ester	A-7 L-Aspartic Acid	A-8 L-Glutamic Acid	A-9 L-Proline	A-10 D-Gluconic Acid	A-11 Dextrin	A-12 Inulin
B-1 D-Cellobiose	B-2 Gentiobiose	B-3 Maltose	B-4 Maltotriose	B-5 D-Melezitose	B-6 D-Melibiose	B-7 Palatinose	B-8 D-Raffinose	B-9 Stachyose	B-10 Sucrose	B-11 D-Trehalose	B-12 Turanose
C-1 N-Acetyl-D-Glucosamine	C-2 α-D-Glucose	C-3 D-Galactose	C-4 D-Psicose	C-5 L-Sorbose	C-6 Salicin	C-7 D-Mannitol	C-8 D-Sorbitol	C-9 D-Arabitol	C-10 Xylitol	C-11 Glycerol	C-12 Tween 80
D-1 Water	D-2 Fumaric Acid	D3 L-Malic Acid	D-4 Succinic Acid Mono-Methyl Ester	D-5 Bromosuccinic Acid	D6 L-Glutamic Acid	D-7 γ-Aminobutyric Acid	D-8 α-Ketoglutaric Acid	D-9 2 -Keto-D-Gluconic Acid	D-10 D-Gluconic Acid	D-11 Dextrin	D-12 Inulin
E-1 D-Cellobiose	E-2 Gentiobiose	E-3 Maltose	E-4 Maltotriose	E-5 D-Melezitose	E-6 D-Melibiose	E-7 Palatinose	E-8 D-Raffinose	E-9 Stachyose	E-10 Sucrose	E-11 D-Trehalose	E-12 Turanose
F-1 N-Acetyl-D-Glucosamine	F-2 D-Glucosamine	F-3 α-D-Glucose	F-4 D-Galactose	F-5 D-Psicose	F-6 L-Rhamnose	F-7 L-Sorbose	F-8 α-Methyl-D-Glucoside	F-9 β-Methyl-D-Glucoside	F-10 Amygdalin	F-11 Arbutin	F-12 Salicin
G-1 Maltitol	G-2 D-Mannitol	G-3 D-Sorbitol	G-4 Adonitol	G-5 D-Arabitol	G-6 Xylitol	G-7 i-Erythritol	G-8 Glycerol	G-9 Tween 80	G-10 L-Arabinose	G-11 D-Arabinose	G-12 D-Ribose
H-1 D-Xylose	H-2 Succinic Acid Mono-Methyl Ester plus D-Xylose	H-3 N-Acetyl-L-Glutamic Acid plus D-Xylose	H-4 Quinic Acid plus D-Xylose	H-5 D-Glucuronic Acid plus D-Xylose	H-6 Dextrin plus D-Xylose	H-7 α-D-Lactose plus D-Xylose	H-8 D-Melibiose plus D-Xylose	H-9 D-Galactose plus D-Xylose	H-10 m-Inositol plus D-Xylose	H-11 1,2-Propanediol plus D-Xylose	H-12 Acetoin plus D-Xylose

Oxidation Tests Assimilation Tests

Procedure Precautions

TO OBTAIN ACCURATE AND REPRODUCIBLE RESULTS, THE INSTRUCTIONS BELOW MUST BE FOLLOWED.

1. Read the entire "Instructions for Use" prior to using the YT Microplates.
2. Pure cultures must be used to obtain identifications. The system is not designed to identify individual yeast strains from within mixed cultures.
3. Culture media selection and performing repeated subculturing prior to testing are very important. Many strains will produce different metabolic patterns depending upon how they are cultured prior to inoculation. Refer to the sections "Step 2. Specimen Preparation" and "Limitations" for details.
4. Sterile components and aseptic techniques must be used in set-up procedures. Contamination will affect results.
5. Disposable glassware should be used to handle all cell suspensions and solutions. Washed glassware may contain trace amounts of soap or detergent that will hamper results.
6. Prewarm the YT-IF or water and the YT Microplates to room temperature before use. Some species may be sensitive to cold shocks.
7. Calibrate your turbidimeter carefully and always prepare your inoculum within the specified density range. Refer to section "Step 3. Inoculum Preparation."
8. Biolog's chemistry contains components sensitive to temperature and light. Dark brown wells in the YT Microplate indicate deterioration of the carbon source. Wells with an inherent light brown, yellow, or pink hue are 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.

Test Procedure

A. Preparations

— Prewarm the YT Microplates and tubes of YT-IF or sterile water to at least 25°C before use.

B. Steps

Step 1. Culture Isolation on Biolog Recommended Media

1. Isolate a pure culture on agar media.
2. Use Biolog recommended media (BUY Agar) and incubate at 26-28°C. All species identifiable with the YT Microplate will grow under these conditions.

Step 2. Specimen Preparation and Characterization

1. Perform a Wet prep or Gram stain to verify it is yeast.
2. Grow the yeast using the recommended conditions. The choice of the agar medium is critical since it must support growth and promote retention of full metabolic activity.
3. The cells must be freshly grown since many strains lose viability and metabolic vigor in the stationary phase. The recommended incubation period for most organisms is 24-48 hours.
4. If insufficient growth is obtained to inoculate the panel, inoculate more than one agar plate.

Step 3. Inoculum Preparation

1. Establish the acceptable turbidity range on your turbidimeter. First, set the transmittance adjustment to 100% by using an uninoculated water tube. Then determine the desired turbidity with the YT Turbidity Standard. Using the Biolog turbidimeter and a 20 mm diameter tube of the Standard, the transmittance value will be approximately 47% with slight variations per Biolog turbidimeter. With other instruments or with other tubes, the transmittance readings may vary substantially.
2. 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.
3. Prepare a uniform suspension as follows: Remove cells from the agar plate with a sterile cotton swab to prevent contamination of the agar medium. Start with isolated colonies, then move 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 into the fluid until the organism becomes a homogenous, clump-free suspension. A sterile transfer pipet may also be used to mix the suspension without creating an aerosol. If clumps remain, let the tube stand for several minutes allowing them to settle.
4. Adjust the inoculum density. Watch as the turbidimeter needle moves toward the acceptable turbidity range. The acceptable turbidity range is defined by the turbidity standard ±2% transmittance. This must be done with precision since it establishes the concentration for the cells and for the redox chemistry. The density can be lowered by adding more water or raised by adding more cells. Note that it is easier to approach the acceptable range by carefully adding cells.
5. Inoculate the cell suspension into the YT Microplate promptly. Some strains lose metabolic activity if held too long (more than 20 minutes) in water without nutrients.

Step 4. Inoculation of the YT Microplate

1. Label the YT Microplate with the organism's name and number.
2. Pour the cell suspension into the multichannel pipet reservoir.
3. If you are using an electronic pipettor, fasten 8 sterile tips securely onto the 8-Channel Repeating Pipettor. Refer to the manufacturer's instructions. Fill the tips and ensure all tips are filling equally. If not, refasten any loose tips.
4. If you are using a manual pipettor, prime the tips by dispensing the first delivery back into the reservoir.
5. Fill all wells with precisely 100 µl. Be careful not to carry over chemicals or splash from one well into another.
6. Cover the YT Microplate with its lid.

Step 5. Incubation

1. Incubate the YT Microplate at 26-28°C.
2. Provide a source of moisture in your incubator to minimize dehydration of the outer wells of the YT Microplate. Placing the YT Microplates in a plastic container with wet paper towels underneath should be sufficient. If you are incubating the YT Microplates in an Odin system, a clear plate seal can be used instead.
3. Incubate plates for 1 to 3 days until a sufficient pattern is formed.

The following table summarizes the testing procedures:

	Microbe type	Yeast
Step 1	Culture medium	BUY
Step 2	Temperature	26-28° C
	Atmosphere	Air
Step 3	Inoculating fluid	YT-IF or sterile water
	Inoculum turbidity	47% T
Step 4	Inoculum per well	100 µl
Step 5	Incubation time	24, 48 and 72 hours

Results

Reading and Interpretation

1. Read the YT Microplates using an Odin™ system, the MicroStation™ Reader, or the MicroLog™ System
2. The color density or turbidity increase in each well is referenced against the negative control wells (A-1 and D-1).
   2.1. All wells optically resembling the negative control wells are scored as "negative" (-).
   2.2. All wells with a noticeable increase in absorbance at 590 nm are scored as "positive" (+).
   2.3. Wells with a minimal 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.
3. "False positive" results are typically defined as purple color or turbidity forming in the control wells (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 YT Microplate can also cause "false positive" color to occur. Refer to the section "Troubleshooting" for details. Some yeast species metabolize carbon sources to form colored (e.g. brown) byproducts. These should be scored as "positive" reactions.
4. For YT 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.

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 identify all species 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 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 be reported 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 report "No identification".

Troubleshooting

If you experience a problem utilizing the YT Microplate, start by rereading the Instructions for Use and review whether you have deviated from the recommended procedures. Then refer to the table below.

If all wells are positive, ensure:	**If all wells are negative, ensure:**
1. You are using a microorganism appropriate for the YT Microplate. 2. You are not carrying over any nutrients from the agar growth medium into the inoculating fluid. 3. Your inoculum density is not excessive – check the calibration of your turbidimeter. 4. Your inoculum is free of clumps. 5. The A-1 and D-1 wells are not under-filled. They are used as reference wells.	1. You are using a microorganism appropriate for the YT Microplate. 2. Your cells are freshly grown and you have used the recommended BUY agar medium. 3. The suspension fluid was prewarmed, prepared appropriately, has the correct pH, and does not contain preservatives. 4. You are handling the cells with all disposable hardware (soap residues are toxic). 5. Your inoculum density is sufficient – check the calibration of your turbidimeter. 6. Your incubation temperature is correct for the organism being tested. 1. The A-1 and D-1 wells are not over-filled. They are used as reference wells.

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. They are available from ATTC®.

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

*The species in parentheses indicates the anticipated ID result from the Biolog database.

Inoculate each yeast following the specified test procedures. When lyophilized or frozen cultures are used, they should be subcultured at least twice before being tested.

Read the YT Microplates 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 procedure. Call Biolog Technical Service if you have any further problems or questions.

Technical Service

For help or to report problems with this product contact Biolog Technical Service during business hours (8 A.M. to 5 P.M. Pacific Time) or contact your local Biolog Distribution Partner.

phone 510-785-2564
email tech@biolog.com

General information, Certificates of Analysis, or SDS documents may 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.