FF Microplate Instructions For Use

For Research Use Only

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Intended Use

The FF Microplate test panel provides a standardized method using 95 biochemical tests to identify and characterize a broad range of fungi including both filamentous (FF) and yeast forms. Biolog's software is used to identify the fungus from its metabolic pattern in the FF Microplate. The Biolog FF Database was designed to identify freshly isolated cultures. Cultures maintained over long periods on agar slants or transferred numerous times often exhibit phenotypic degeneration. They may no longer exhibit physiological responses characteristic of the species. The FF 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 different compounds. The test panel yields a phenotypic fingerprint of the microorganism, with a characteristic pattern of reddish-orange wells and turbidity changes for making a species level identification.[1,2]

All necessary nutrients and biochemicals are prefilled and dried into the 96 wells of each plate. Iodonitrotetrazolium violet is used as a redox dye to colorimetrically indicate the mitochondrial activity stimulated during the oxidation of certain carbon sources.

The isolate to be identified is grown on agar medium and later suspended in an inoculating fluid[3] (FF-IF)[4] with gelling properties at the recommended cell density. Then the cell suspension, typically containing spores and mycelial fragments, is inoculated into the FF Microplate at 100 µl per well. Each well appears colorless when inoculated. During incubation in wells containing a utilized carbon source, either or both of the following changes occur:

1. Increased mitochondrial activity, forming a reddish-orange color and an increase in optical density at 490nm.
2. Increased growth, leading to turbidity, and an increase in optical density at 490 and 750 nm. The reference well for color and turbidity (A-1) contains no carbon sources.

The FF Microplates are incubated for 1 to 4 days. The pattern of reddish-orange and turbid wells is read with an Odin™ or MicroStation™ Reader at both 490 and 750 nm to detect and quantify both color and turbidity responses. Biolog's software 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 FF Microplates for damage from shipping. *DO NOT USE THE FF Microplates IF THE PACKAGING INDICATES SIGNS OF DAMAGE*.
2. Store the FF Microplates at 2-8° C inside their pouches to preserve full shelf life.
3. Ensure each FF Microplate is within the expiration date printed on each pouch. *DO NOT USE THE FF Microplates AFTER THE EXPIRATION DATE*.

Materials Required for Test Procedure

Materials Included

1. 10 Biolog FF Microplates (Biolog Catalog #1006)

Materials Not Included

1.	2% Malt Extract Agar (2% ME Agar)	2% Malt Extract Agar (20 g Malt Extract dehydrated medium, Oxoid® Catalog #LP0039B; 18 g Bacteriological Grade Agar; 1 L distilled water) or plated 2% Malt Extract Agar (Biolog Catalog #71106).
2.	FF-Inoculating Fluid (FF-IF)	Sterile disposable glass (borosilicate) test tubes, 20ml capacity (20 x 150 mm) containing 16 ml of sterile "gelling" inoculating fluid (0.25% Phytagel, 0.03% Tween 40, Biolog Catalog #72106)[3,4].
3.	LongSwabs™	Sterile 7-inch disposable cotton-tipped swabs (Biolog Catalog #3023).
4.	Streakerz™	Sterile 6-inch tapered wooden streaking sticks (Biolog Catalog #3026).
5.	Transfer Pipets	Sterile disposable 9-inch transfer pipets (Biolog Catalog #3019).
6.	Reservoirs	Sterile disposable reservoirs for multichannel pipettor (Biolog Catalog #3102).
7.	Pipettor	8-Channel Repeating Pipettor (Biolog Catalog #3711).
8.	Pipettor Tip	Sterile disposable filter tips (Biolog Catalog #3203).
9.	Microplate Lid	Gamma irradiated Microplate lids (Biolog Catalog #30201).
10.	Turbidimeter	Biolog Catalog #3587
11.	Turbidity Standard	FF (Biolog Catalog # 3426).
12.	Incubator	26°C.
13.	Incubation container	A plastic box with lid or a plastic bag to prevent excessive evaporation in the FF Microplate wells during incubation.

FF Microplate Map

A-1 Water	A-2 Tween 80	A-3 N-Acetyl-D-Galactosamine	A-4 N-Acetyl-D-Glucosamine	A-5 N-Acetyl-D-Mannosamine	A-6 Adonitol	A-7 Amygdalin	A-8 D-Arabinose	A-9 L-Arabinose	A-10 D-Arabitol	A-11 Arbutin	A-12 D-Cellobiose
B-1 α-Cyclodextrin	B-2 β-Cyclodextrin	B-3 Dextrin	B-4 i-Erythritol	B-5 D-Fructose	B-6 L-Fucose	B-7 D-Galactose	B-8 D-Galacturonic Acid	B-9 Gentiobiose	B-10 D-Gluconic Acid	B-11 D-Glucosamine	B-12 α-D-Glucose
C-1 Glucose-1-Phosphate	C-2 Glucuronamide	C-3 D-Glucuronic Acid	C-4 Glycerol	C-5 Glycogen	C-6 m-Inositol	C-7 2-Keto-D-Gluconic Acid	C-8 α-D-Lactose	C-9 Lactulose	C-10 Maltitol	C-11 Maltose	C-12 Maltotriose
D-1 D-Mannitol	D-2 D-Mannose	D-3 D-Melezitose	D-4 D-Melibiose	D-5 α-Methyl-D-Galactoside	D-6 β-Methyl-D-Galactoside	D-7 α-Methyl-D-Glucoside	D-8 β-Methyl-D-Glucoside	D-9 Palatinose	D-10 D-Psicose	D-11 D-Raffinose	D-12 L-Rhamnose
E-1 D-Ribose	E-2 Salicin	E-3 Sedohep-tulosan	E-4 D-Sorbitol	E-5 L-Sorbose	E-6 Stachyose	E-7 Sucrose	E-8 D-Tagatose	E-9 D-Trehalose	E-10 Turanose	E-11 Xylitol	E-12 D-Xylose
F-1 γ-Amino-butyric Acid	F-2 Bromosuccinic Acid	F-3 Fumaric Acid	F-4 β-Hydroxy-butyric Acid	F-5 γ-Hydroxy-butyric Acid	F-6 p-Hydroxyphenyl-acetic Acid	F-7 α-Keto-glutaric Acid	F-8 D-Lactic Acid Methyl Ester	F-9 L-Lactic Acid	F-10 D-Malic Acid	F-11 L-Malic Acid	F-12 Quinic Acid
G-1 D-Saccharic Acid	G-2 Sebacic Acid	G-3 Succinamic Acid	G-4 Succinic Acid	G-5 Succinic Acid Mono-Methyl Ester	G-6 N-Acetly-L-Glutamic Acid	G-7 Alaninamide	G-8 L-Alanine	G-9 L-Alanyl-Glycine	G-10 L-Asparagine	G-11 L-Aspartic Acid	G-12 L-Glutamic Acid
H-1 Glycyl-L-Glutamic Acid	H-2 L-Ornithine	H-3 L-Phenylalanine	H-4 L-Proline	H-5 L-Pyroglutamic Acid	H-6 L-Serine	H-7 L-Threonine	H-8 2-Amino Ethanol	H-9 Putrescine	H-10 Adenosine	H-11 Uridine	H-12 Adenosine-5'-Monophosphate

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 FF Microplate.
2. A pure culture must be used to obtain an identification. The system is not designed to identify fungi within mixed cultures.
3. Strains will produce different metabolic patterns depending upon how they are grown prior to inoculation. Refer to the sections titled "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 FF-IF and the FF Microplates to room temperature before use.

7. Calibrate your turbidimeter carefully and always prepare your inoculum within the specified density range. Refer to section "Step 3. Inoculum Preparation".
8. Handle all fungi in a safe manner. Special care must be taken to avoid personnel exposure to and equipment cross contamination from air-borne spores (conidia). Laboratory safety guidelines have been published by several organizations, including the Centers for Disease Control, the National Institutes of Health, the Medical Research Council of Canada, and the World Health Organization. Biolog recommends that you follow the guidelines in these publications. The use of a type II biological safety cabinet is recommended. Sterile replacement Microplate lids will help reduce contamination within the incubator and plate reader you are using.
9. Biolog's chemistry contains components that are sensitive to temperature and light.

Test Procedure

A. Preparations

- Invert or gently mix tubes of FF-IF to resuspend gelling agent prior to use.
- Prewarm the FF Microplates and tubes of FF-IF 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 (2% ME Agar) or prepared media from Biolog (Biolog Catalog #71106) and incubate at 20-26°C, as appropriate to the organism studied.

Step 2. Specimen Preparation and Characterization

A. Filamentous Fungi (FF)

1. Culture the fungus on 2% ME Agar, a medium that promotes sporulation.
2. Incubate the agar plates under ambient daylight or near-ultraviolet lighting until sporulation occurs, typically 5-10 days at 26°C.
   2.1. Many fungi produce sufficient conidial inoculum under ambient daylight. Some groups, e.g. plant pathogenic fungi, such as *Fusarium* species, may require incubation under near-UV lighting to enhance sporulation.

B. Yeast

1. Culture yeast on 2% ME Agar and incubate 24–48 hours at 26°C.

Step 3. Inoculum Preparation

1. Establish the acceptable turbidity range on your turbidimeter. First, set the transmittance adjustment to 100% by using an uninoculated FF-IF tube. Then, determine the desired turbidity with the FF Turbidity Standard. Using the Biolog turbidimeter and a 20mm diameter tube of the Standard, the transmittance value will be approximately 75% with slight variations per Biolog Turbidimeter. With other instruments or tubes, the transmittance readings may vary substantially.
2. Blank the turbidimeter (transmittance = 100%) with a clean tube containing uninoculated FF-IF. 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:
   3.1. In a type II biological safety hood, remove spores or conidia from the agar plate with a sterile swab to prevent contamination from the agar medium.
   3.2. Yeast suspensions may be set up on the bench.

3.3. Dip the swab into the FF-IF to moisten it. Transfer cells onto the swab by rolling it across the sporulating areas (do not slide across). Twirl and press the swab against the inside surface of the tube on the dry glass above the fluid line to dislodge and separate conidia and release cells. Gently, but thoroughly, mix the tube. USE CARE TO NOT INTRODUCE AIR BUBBLES AS THEY WILL INTERFERE WITH THE ABSORBANCE READING. Check the suspension for clumps and air bubbles. If they are present, 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 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 inoculating fluid 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 FF Microplate promptly. Some strains lose metabolic activity if held too long (no more than 10 minutes) in inoculating fluid without nutrients.

Step 4. Inoculation of the FF Microplate

1. Label the FF Microplate with the organism name/number.
2. Pour the cell suspension into the multichannel pipet reservoir.
3. Fasten 8 sterile filter tips securely onto the 8-Channel Repeating Pipettor.
4. Fill the tips and ensure all tips are filling equally. If not, refasten any loose tips.
5. If you are using a manual pipettor, prime the tips by dispensing the first delivery back into the reservoir.
6. Fill all wells with precisely 100 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.
7. The inoculating fluid will form a soft gel shortly after inoculation.
8. Cover the FF Microplate with its lid.

Step 5. Incubation of the FF Microplate

1. Place the FF Microplate in a plastic container or plastic bag. Do not add a source of moisture to this container, as mycelial growth could contaminate the exterior of the FF Microplate. If you are incubating the FF Microplates in an Odin system, a clear plate seal can be used instead of a plastic bag.
2. Incubate the FF Microplates for 1-4 days, at 26°C (or up to 10 days for osmophilic or extremely slow growing fungi).

The following table summarizes the testing procedures:

	Microbe type	Filamentous Fungi (FF)	Yeast
Step 1	Culture medium	2% ME	
	Temperature	26° C	
Step 2	Atmosphere	Air	
	Wet prep results	Hyphal elements	Yeast cells
	Culture Incubation time	5-10 days	2 days
Step 3	Inoculating fluid	FF-IF	
	Turbidity standard	FF	
	Inoculum turbidity	75% T	
Step 4	Inoculum per well	100 µl	
Step 5	Incubation times, if incubated outside of an Odin system	24, 48, 72, and 96 hours *EXCEPTION:* up to 10 days for osmophilic, or extremely slow growing fungi.	

Note: We recommend that filamentous fungi be manipulated in a biological safety cabinet.

Results

Reading and Interpretation

1. Read the FF Microplates using an Odin™ system, the MicroStation™ Reader, or the MicroLog™ System. Condensation on the lid interferes with accurate readings. If condensation is present, remove the Microplate lid (in a type II biological safety hood) and replace it with a sterile lid to prevent contamination. Do not place Microplate lids onto unsterile surfaces or set them upside down as this can introduce contamination. Refer to the User Guide of the specific system or software you are using. Biolog software will calculate threshold values to determine -, /, and + wells.
2. In some species it is normal for the A-1 control well to develop a distinct color caused by germination of spores or conidia (in the absence of carbon sources). Biolog software can accommodate these "false-positive" reactions.
3. Most species give clearly discernible "positive" color reactions. However, it is normal for the "positive" color reactions of certain genera to be light or faint reddish-orange.
4. The following table shows the similarity index considered acceptable per length of incubation:

Length of incubation	Similarity index
1 day	≥ 0.90
2 days	≥ 0.70
3 days	≥ 0.65
4 days	≥ 0.60

   These four threshold values give comparable levels of accuracy.

5. The Food and Air Databases contain the patterns for all common food and air-borne fungi, including yeasts, Aspergillus, Penicillium, and other genera.
6. Read each FF Microplate daily until an identification is obtained, usually within 4 days. Once an identification is obtained, discontinue reading that FF Microplate. Osmophilic and other slow growing fungi may require an additional 7 days.

7. Verify identifications by comparing the macroscopic and microscopic morphologies against the photo library (where image is available). If the identification of the first choice does not match the images, compare the culture morphology to the second and third choices' images.
8. If no identification occurs after 4 days, continue checking macroscopic and microscopic morphology of the first 3 choices.
9. Use the correct databases. If the isolate came from an air sample, the pattern should be compared to the Air Database. Similarly, a contaminant from a food sample should be compared to the Food Database.
10. The genus databases may be used when the isolate has been identified to the corresponding genus. The genus databases are more inclusive and contain species not commonly foodborne or airborne.
11. The yeast suspension contains live yeast cells and may produce a pattern in 24 hours. In contrast, the filamentous fungi suspension contains primarily spores or conidia, which must germinate and grow to produce a pattern. The time necessary for the spores to germinate is variable, increasing the variation seen in 24 hour patterns and decreasing the reliability of 24 hour reads for fungi.
    11.1. FF identification workflow: Read after 24 hours and daily until identification is called.

Read daily	ID call ⇒ Verify macro/microscopically (first 3 choices)
	No ID ⇒ Check macro/microscopically (first 3 choices) and Re-incubate FF Microplate,
Read 96 hours	ID call ⇒ Verify macro/microscopically (first 3 choices)
	No ID ⇒ Check macro/microscopically (first 3 choices)

Note: Osmophiles, xerophiles, and other extremely slow growing fungi may require 7 days incubation to show a positive result.

Performance Characteristics

The FF Microplate performance characteristics have been determined by establishing a database from a large collection of stock and freshly isolated cultures. The database is designed to give identifications of all species in the database, in accordance with current standards of taxonomic nomenclature. TO OBTAIN ACCURATE AND REPRODUCIBLE RESULTS, ALL PROCEDURES AND RECOMMENDATIONS IN THE INSTRUCTIONS FOR USE MUST BE FOLLOWED PRECISELY.

Limitations

The FF Microplate is designed to identify and/or characterize freshly isolated, pure cultures of sporulating fungi. The software will only recognize patterns on the FF Microplate for species in the current database. Other fungal species will be reported with the message "No identification." Atypical strains may also yield a similarity index that is less than 0.6 at 4 days and therefore will report "No identification." Fungi that have been repeatedly transferred (e.g. old type cultures) may have altered metabolic properties and may read as "No identification."

Troubleshooting

If you experience a problem utilizing the FF 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 an uncontaminated pure culture. Non-fungal species may produce all positive wells. 2. You are not carrying over any nutrients into the inoculating fluid. 3. Your inoculum density is not excessive—check the calibration of your turbidimeter. 4. The A-1 well is not under-filled. It is used as a reference well.	1. You are using a microorganism that is appropriate for the FF Microplate (sporulating fungus, food, or airborne yeast). 2. Your cultures are freshly grown on the recommended 2% ME agar medium. 3. The inoculating fluid was prepared correctly and does not contain preservatives. 4. Your incubation temperature is ~26°C. 5. Your inoculum density is sufficient – check the calibration of your turbidimeter. 6. The A-1 well is not over-filled. It is used as a reference well.

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 FF Microplate use the 3 strains specified below. They are available from ATCC®.

*1. Galactomyces geotrichum (Geotrichum candidum or Galactomyces candidus)**	ATCC 34614™
2. Aspergillus niger	ATCC 16888™
3. Penicillium chrysogenum	ATCC 10106™

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

Inoculate each culture following the specified test procedures.

Read the FF Microplates daily until an identification is called. 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 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.

[3] Biolog, Inc., US Patent # 5,627,045.

[4] Prepare FF-IF as follows. Bring distilled water to a boil. Add Phytagel at 2.5 g/L (Sigma® Chemical, cat # P8169) and Tween 40 at 0.3 g/L (Sigma Chemical, cat # P1504) and stir without further heating for about 10 minutes until all components are dissolved and the solution becomes clear. Dispense 16 ml into capped, disposable borosilicate tubes (20 x 150 mm) and sterilize by autoclaving.

[5] Biolog recommends using these components when making 2%ME Agar to ensure consistent results.

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