BIOLOG

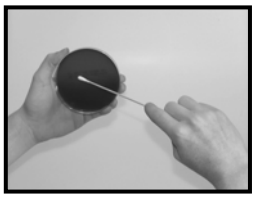
Actinomycetes & Fungi Test Panel

SF-N2 MicroPlate™*
SF-P2 MicroPlate™*

and other sporulating and filamentous microorganisms
for research use only

STEP 1

Isolate a pure culture under conditions that promote sporulation

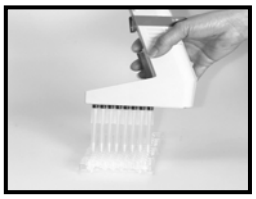


STEP 2

Prepare inoculum at specified cell density

STEP 3

Inoculate and incubate MicroPlate



STEP 4

Read MicroPlate and determine metabolic properties

The SF-N2 and SF-P2 MicroPlates can be used for easy and rapid metabolic testing of Sporulating and Filamentous (SF) microorganisms such as actinomycetes and fungi. Traditionally, testing of sporulating and filamentous microbes has been very labor intensive, difficult, and slow. Because they can form hydrophobic mycelial filaments, these organisms have a strong tendency to form clumps and adhere to surfaces, making them difficult to handle. Furthermore, because they can form spores, germinate, and then undergo complex life cycles, it is very difficult to get cultures which perform consistently in metabolic and biochemical testing regimes.

Testing of SF microorganisms in SF-N2 and SF-P2 MicroPlates is very simple and uses virtually the same protocol and equipment used for testing coccoid and rod-shaped bacteria and yeast in the MicroLog™ System. The four basic steps are the same (as shown above), but with a few simple modifications. Special culture media are used for these microorganisms to promote sporulation. Inocula are prepared at a lower density, approximately one tenth the density normally used. Inocula are prepared in a gel-forming colloid instead of in water or saline. The suspensions are inoculated at 100µl per well (instead of 150µl per well) and SF-N2 and SF-P2 MicroPlates are used instead of GN2 and GP2 MicroPlates. The SF-N2 and SF-P2 MicroPlates are identical to the GN2 or GP2 MicroPlates (see plate layouts on the reverse side) but they do not contain the tetrazolium redox dye which is toxic to many of these species. After inoculation, the SF MicroPlates are typically incubated at an appropriate low temperature (e.g. 26°C) for several days (e.g. 1 to 5).

*US Patent # 5,627,045.

The inoculated microorganism grows in wells containing carbon sources that it can utilize, forming cloudy turbidity in those wells as compared to the A-1 reference well. The colloid used to suspend the cells enhances growth and keeps the cells uniformly suspended. After sufficient incubation, "positive" reactions are determined as increased cloudiness or turbidity. Many of these microorganisms also produce distinct characteristics such as pigments in wells with particular carbon sources.

Recommended testing method*:
Grow the strains at 26°C for 2 to 4 days on an appropriate agar medium to promote sporulation. For actinomycetes it often helps to increase the agar concentration to a higher than normal level of agar (e.g. 25g/L). Harvest cells/spores/mycelial fragments from the surface of the agar medium by simply rubbing a moistened cotton swab across the surface of the cell mass. First, prepare a suspension in 13.5 ml of a gel forming colloid (e.g. 0.2% Gellan Gum, Sigma Chemical Co. product # P8169 or Carageenan Type II, Sigma Chemical Co. product # C1138) sterilized by autoclaving for 20 – 30 minutes. Prepare a cell suspension with a density corresponding to 60% transmittance (an OD590 of about 0.22) in the Biolog turbidimeter using a 20 mm diameter tube. Let the tube stand for several minutes and allow clumps to settle to the bottom. Dilute tenfold (add 1.5 ml of this suspension) into a second tube containing 13.5 ml of colloid. Inoculate the SF-N2 or SF-P2 MicroPlate with 100µl per well. Incubate for 1 to 5 days at 26°C until a stable pattern has formed. Read the results by eye or using the Biolog MicroStation™ Reader.

The entire testing procedure takes 3 to 7 days in elapsed time and about five minutes of hands-on labor.

SF-N2 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

SF-P2 MicroPlate™

A1 Water	**A2** α-Cyclodextrin	**A3** β-Cyclodextrin	**A4** Dextrin	**A5** Glycogen	**A6** Inulin	**A7** Mannan	**A8** Tween 40	**A9** Tween 80	**A10** N-Acetyl-D-Glucosamine	**A11** N-Acetyl-β-D-Mannosamine	**A12** Amygdalin
B1 L-Arabinose	**B2** D-Arabitol	**B3** Arbutin	**B4** D-Cellobiose	**B5** D-Fructose	**B6** L-Fucose	**B7** D-Galactose	**B8** D-Galacturonic Acid	**B9** Gentiobiose	**B10** D-Gluconic Acid	**B11** α-D-Glucose	**B12** m-Inositol
C1 α-D-Lactose	**C2** Lactulose	**C3** Maltose	**C4** Maltotriose	**C5** D-Mannitol	**C6** D-Mannose	**C7** D-Melezitose	**C8** D-Melibiose	**C9** α-Methyl-D-Galactoside	**C10** β-Methyl-D-Galactoside	**C11** 3-Methyl-D-Glucose	**C12** α-Methyl-D-Glucoside
D1 β-Methyl-D-Glucoside	**D2** α-Methyl-D-Mannoside	**D3** Palatinose	**D4** D-Psicose	**D5** D-Raffinose	**D6** L-Rhamnose	**D7** D-Ribose	**D8** Salicin	**D9** Sedoheptulosan	**D10** D-Sorbitol	**D11** Stachyose	**D12** Sucrose
E1 D-Tagatose	**E2** D-Trehalose	**E3** Turanose	**E4** Xylitol	**E5** D-Xylose	**E6** Acetic Acid	**E7** α-Hydroxybutyric Acid	**E8** β-Hydroxybutyric Acid	**E9** γ-Hydroxybutyric Acid	**E10** p-Hydroxy-phenylacetic Acid	**E11** α-Ketoglutaric Acid	**E12** α-Ketovaleric Acid
F1 Lactamide	**F2** D-Lactic Acid Methyl Ester	**F3** L-Lactic Acid	**F4** D-Malic Acid	**F5** L-Malic Acid	**F6** Pyruvic Acid Methyl Ester	**F7** Succinic Acid Mono-Methyl Ester	**F8** Propionic Acid	**F9** Pyruvic Acid	**F10** Succinamic Acid	**F11** Succinic Acid	**F12** N-Acetyl-L-Glutamic Acid
G1 L-Alaninamide	**G2** D-Alanine	**G3** L-Alanine	**G4** L-Alanyl-Glycine	**G5** L-Asparagine	**G6** L-Glutamic Acid	**G7** Glycyl-L-Glutamic Acid	**G8** L-Pyroglutamic Acid	**G9** L-Serine	**G10** Putrescine	**G11** 2,3-Butanediol	**G12** Glycerol
H1 Adenosine	**H2** 2'-Deoxy Adenosine	**H3** Inosine	**H4** Thymidine	**H5** Uridine	**H6** Adenosine-5'-Monophosphate	**H7** Thymidine-5'-Monophosphate	**H8** Uridine-5'-Monophosphate	**H9** D-Fructose-6-Phosphate	**H10** α-D-Glucose-1-Phosphate	**H11** D-Glucose-6-Phosphate	**H12** D-L-α-Glycerol Phosphate

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