

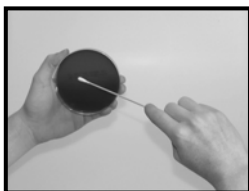
SF-N2 MicroPlate™*

SF-P2 MicroPlate™*

and other sporulating and filamentous microorganisms
for research use only

STEP 1

Isolate a pure culture
under conditions that
promote sporulation



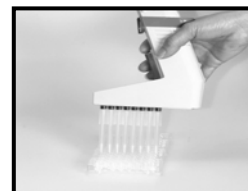
STEP 2

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 l-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 Turannose	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-phenylacetic 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-Pyroglytamic 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 Phenylethylamine	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 Turannose	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-Pyroglytamic 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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