m FC Agar • m FC Broth Base
Rosolic Acid

Intended Use
m FC Agar and m FC Broth Base are used with Rosolic Acid in cultivating and enumerating fecal coliforms by the membrane filter technique at elevated temperatures.

Summary and Explanation
Geldreich et al.[1] formulated a medium to enumerate fecal coliforms (MFC) using the membrane filter (MF) technique without prior enrichment. Fecal coliforms (i.e., those found in the feces of warm-blooded animals) are differentiated from coliforms from environmental sources by their ability to grow at 44.5 ± 0.5°C.[2]

Many "standard methods" membrane filtration procedures specify m FC medium for testing water.[2-4] The American Public Health Association (APHA) specifies m FC medium and incubation at 44.5 ± 0.5°C in the fecal coliform membrane filter procedure, the delayed-incubation fecal coliform procedure and the two-layer agar method for recovering injured fecal coliforms.[2] AOAC International specifies m FC Agar for detecting total coliforms and fecal coliforms in foods.[3]

The U. S. Environmental Protection Agency specifies using m FC medium in fecal coliform methods for testing water by the direct MF method or the delayed-incubation MF method.[4,5]

Principles of the Procedure
m FC Agar and m FC Broth Base contain peptones as sources of carbon, nitrogen, vitamins and minerals.Yeast extract supplies B-complex vitamins that stimulate bacterial growth. Lactose is a carbohydrate. Bile Salts No. 3 inhibits growth of gram-positive bacteria. m FC Agar contains agar as the solidifying agent. The differential indicator system combines aniline blue and rosolic acid.

Colonies of fecal coliforms are blue; non-fecal coliforms and other organisms are gray to cream-colored.

Formulae
Difco™ m FC Agar
Approximate Formula* Per Liter

Tryptose	10.0	g
Proteose Peptone No. 3	5.0	g
Yeast Extract	3.0	g
Lactose	12.5	g
Bile Salts No. 3	1.5	g
Sodium Chloride	5.0	g
Agar	15.0	g
Aniline Blue	0.1	g

Difco™ m FC Broth Base
Consists of the same ingredients without the agar.

Difco™ Rosolic Acid
Rosolic Acid	1	g/vial

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product
Difco™ m FC Agar
1. Suspend 52 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Add 10 mL of a 1% solution of Rosolic Acid in 0.2N NaOH. Continue heating for 1 minute. DO NOT AUTOCLAVE.
4. If necessary, adjust to pH 7.4 with 1N HCl.
5. Test samples of the finished product for performance using stable, typical control cultures.

Difco™ m FC Broth Base
1. Suspend 3.7 g of the powder in 100 mL of purified water.
2. Add 1 mL of a 1% solution of Rosolic Acid in 0.2N NaOH.
3. If necessary, adjust to pH 7.4 with 1N HCl.
4. Heat to boiling. DO NOT AUTOCLAVE.
5. Cool before dispensing.
6. Test samples of the finished product for performance using stable, typical control cultures.

Difco™ Rosolic Acid
Prepare a 1% solution, dissolving 1 g in 100 mL of 0.2N NaOH.

User Quality Control

Identity Specifications

Difco™ m FC Agar

Dehydrated Appearance:	Beige with a slight blue tint to blue, free-flowing, homogeneous.
Solution:	5.2% solution, soluble in purified water upon boiling. Without 1% Rosolic Acid: blue, very slightly to slightly opalescent, may have a slight precipitate. With 1% Rosolic Acid: cranberry red, slightly opalescent, may have a slight precipitate.
Prepared Appearance:	Without 1% Rosolic Acid—Blue, slightly opalescent, may have slight precipitate. With 1% Rosolic Acid—Cranberry red, slightly opalescent, may have slight precipitate.
Reaction of 5.2% Solution at 25°C:	pH 7.4 ± 0.2 (without 1% Rosolic Acid)

Difco™ m FC Broth Base

Dehydrated Appearance:	Beige with a slight blue tint to blue, free-flowing, homogeneous.
Solution:	3.7% solution, soluble in purified water upon boiling. Solution is blue, slightly opalescent, may have a precipitate.
Prepared Appearance:	Without 1% Rosolic Acid—Blue, slightly opalescent, may have a very fine precipitate. With 1% Rosolic Acid—Cranberry red, slightly opalescent, may have a slight precipitate.
Reaction of 3.7% Solution at 25°C:	pH 7.4 ± 0.2 (without 1% Rosolic Acid)

Difco™ Rosolic Acid

Dehydrated Appearance:	Dark reddish-brown with metallic green particles, free-flowing, fine crystalline powder.
Solution:	1.0% solution, soluble in 0.2N NaOH. Solution is deep red, clear to very slightly opalescent.

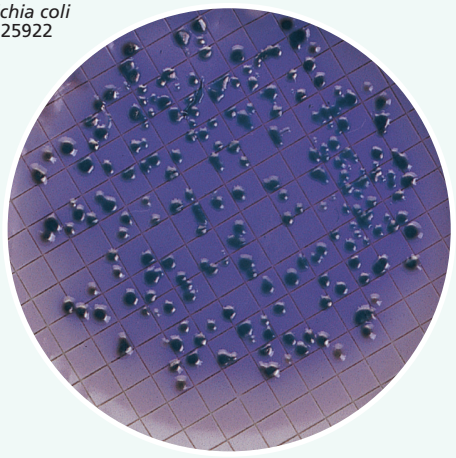
Cultural Response

Difco™ m FC Agar or m FC Broth Base

Prepare the medium per label directions with 1% Rosolic Acid. Using the membrane filter technique, inoculate and incubate plates at 44.5 ± 0.5°C for 24 ± 2 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR
Enterococcus faecalis	19433	10^3-2×10^3	Marked to complete inhibition	–
Escherichia coli	25922	20-80	Good	Blue

Escherichia coli ATCC™ 25922



Procedure

Difco™ m FC Agar

1. Prepare the agar medium from the dehydrated base according to the label directions and with the addition of the Rosolic Acid solution.
2. Pour molten agar, previously cooled to 45-50°C into special tight-fitting plastic dishes and allow to harden.
3. Roll the membrane filter used to collect the water sample onto the surface of the agar, so as to avoid the formation of air bubbles between the filter and the agar surface.
4. Place the dishes in plastic bags and incubate, by immersion, in a water bath at 44.5 ± 0.2°C for 24 ± 2 hours.

Difco™ m FC Broth

1. Prepare the broth medium from the dehydrated base according to the label directions and with the addition of the Rosolic Acid solution.
2. Add 2 mL of the cooled broth to sterile absorbent pads in special tight-fitting plastic dishes.
3. Roll the membrane filter used to collect the water sample onto the moistened absorbent pad, so as to avoid the formation of air bubbles between the filter and the pad.
4. Place the dishes in plastic bags and incubate, by immersion, in a water bath at 44.5 ± 0.2°C for 24 ± 2 hours.

Expected Results

Colonies of fecal coliforms will be various shades of blue. Non-fecal coliforms are gray to cream-colored.

Limitation of the Procedure

A few non-fecal coliform colonies may be observed on m FC media due to the selective action of the elevated temperature and the addition of the Rosolic Acid. It may be useful to elevate the temperature to 45 ± 0.2°C to eliminate *Klebsiella* strains from the fecal coliform group.[6]

References

1. Geldreich, Huff and Best. 1965. J. Am. Water Works Assoc. 57:208.
2. Eaton, Rice and Baird (ed). 2005. Standard methods for the examination of water and wastewater, 21st ed., online. American Public Health Association, Washington, D.C.
3. Horwitz (ed.). 2007. Official methods of analysis of AOAC International, 18th ed., online. AOAC International. Gaithersburg, Md.
4. U.S. Environmental Protection Agency. 1992. Manual for the certification of laboratories analyzing drinking water. EPA-814B-92-002. Office of Ground Water and Technical Support Division, USEPA, Cincinnati, Ohio.
5. Bordner, Winter and Scarpino (ed.). 1978. Microbiological methods for monitoring the environment: water and wastes. Publication EPA-600/8-78-017. Environmental Monitoring and Support Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, Ohio.
6. Eaton, Clesceri and Greenberg (ed.). 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.

Availability

Difco™ m FC Agar

AOAC CCAM EPA SMWW

Cat. No.	267710	Dehydrated – 100 g
	267720	Dehydrated – 500 g

Difco™ m FC Broth Base

EPA SMWW

Cat. No.	288320	Dehydrated – 100 g
	288330	Dehydrated – 500 g

Difco™ Rosolic Acid

Cat. No.	232281	Vial – 6 × 1 g