

# m-FC AGAR

- For in vitro use only -

Catalogue No. PF25, 26 & 27

Our m-FC Agar is a selective membrane filtration medium used for the cultivation and enumeration of fecal coliforms.

Fecal coliforms are coliforms found in the feces of warm-blooded animals, and can be differentiated from other environmental coliforms based on their ability to grow at an elevated temperature of 44.5°C. The higher incubation temperature and the addition of rosolic acid make the medium selective.

Our m-FC Agar is based on the formulation reported by Geldrich et al. in 1965. Many regulating agencies recommend the use of m-FC Agar in enumerating fecal coliforms using the membrane filter technique. The American Public Health Association (APHA) and Environmental Protection Agency (EPA) specify the use of m-FC Agar for the testing of water. Also, the Association of Official Analytical Chemists (AOAC) and Health Canada (HPB) recommend the use of this medium for the enumeration of fecal coliforms in food.

The sample size will vary depending on the expected bacterial density. An ideal sample volume will yield between 20 and 80 colonies.

## Formula per Litre of Medium

Meat Peptone (Tryptose)	10.0 ຊ
Polypeptone	5.0 g
Yeast Extract	3.0 g
Lactose	12.5 g
Sodium Chloride	5.0 g
Bile Salts No. 3	1.5 g
Aniline Blue	0.1 g
Rosolic Acid 1%	10.0 mL
Agar	15.0 g

 $pH7.4 \pm 0.2$ 

## **Recommended Procedure**

#### I. APHA Procedure (Water)

- 1. Allow m-FC Agar plates to warm to room temperature prior to use.
- Using an appropriate sterile filtration unit, filter an appropriate volume of the water sample.
- Using aseptic technique, slowly roll the membrane filter onto the surface of the m-FC Agar to minimize air bubbles. If patches of air are observed carefully lift and reposition the filter.
- 4. Place inoculated plates in waterproof plastic bags or seal in a inverted position, agar side up. Submerge and incubate plates in a water bath for 24 ± 2 hours at 44.5 ± 0.2°C. Anchor plates below the surface of the water to ensure adequate heat distribution.
- After the incubation period remove plates and enumerate typical and atypical coliform colonies using a dissecting microscope or other suitable optical device.

#### II. HPB Procedure (MFLP-55)

Enumeration of Faecal Coliforms in Foods by the HGMF Method

- 1. Allow m-FC Agar plates to warm to room temperature prior to use.
- Prepare a 1:10 dilution of the food by aseptically adding 10 g or mL into 90 mL of sterile peptone water (Dalynn BP45-90) in a stomacher bag or other appropriate sterile bottle.
- 3. Use the 1:10 dilution to prepare further dilutions of the sample if required.
- 4. Stomach for 1 minute. If the sample is contained in a bottle, shake the bottle 25 times through a 30 cm arc in approximately 7 seconds.

- 5. Prior to filtering the sample, agitate the contents of each bag or bottle to resuspend any material that may have settled out.
- 6. Follow manufacturer's instructions for use of the filtration apparatus.
- 7. Aseptically pipette 1.0 mL of the required dilution, open the filter valve until all the liquid has passed through the filter unit.
- 8. Aseptically remove the HGMF and transfer it onto to a m-FC Agar (without rosolic acid) plate. Slowly roll it onto the surface of the agar to minimize trapping air bubbles.
- 9. Incubate plates in an inverted position in stacks of no more than two at 44.5°C for 24 ± 2 hours. Use a water bath or a heat-sink incubator for incubation.
- 10. After the incubation period, enumerate fecal coliform colonies using an automated HGMF Interpreter, or manually using a Linecounter.

## **Interpretation of Results**

Fecal coliforms are able to ferment the lactose contained in the media at the elevated temperatures to form blue colonies. Non-fecal coliforms are normally grey or cream-colored.

Enumerate plates as prescribed by APHA or other appropriate literary reference. An ideal sample will yield 20 to 60 colonies per 50 or 60-mm dish. The APHA recommends verification of typical blue colonies and any atypical gray or green colonies.

- The membrane filtration technique may be inappropriate for testing high turbidity waters or where large numbers of noncoliforms are present
- To meet the need for greater temperature control, use a water bath or heat-sink incubator. Standard incubators may pose a potential problems due to static air and heat layering within the chamber

#### **Quality Control**

After checking for correct pH, colour, depth, and sterility, the following organisms are used to determine the growth performance of the completed medium.

Organism	<b>Expected Result</b>
Escherichia coli ATCC 25922	Growth, blue colonies
Enterobacter aerogenes ATCC 12453	Growth, gray to cream colored colonies
Enterococcus faecalis ATCC 29212	Inhibition

## Storage and Shelf Life

Our m-FC Agar should be protected from light and stored at 4°C to 8°C. The medium side should be uppermost to prevent excessive accumulation of moisture on the agar surface. Under these conditions this medium has a shelf life of 2 weeks from the date of manufacture.

#### **Ordering Information**

Cat#	Description	Format
PF25	m-FC Agar (Tight-fitting) [Membrane 11x50-mm plate]	10/pkg
PF26	m-FC Agar [Standard 15x100-mm plate]	10/pkg
PF27	m-FC Agar [Membrane 15x60-mm plate]	10/pkg

#### References

- Geldreich EE, Clark HF, Huff CB, Best LC. Fecal-coliform-organism medium for the membrane filter technique. J Amer Water Works Assoc 1965; 57:208.
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- Sartory DP. Membrane filtration fecal coliform determinations with unmodified and modified M-FC medium. Water Sa 1980; 6:113.
- 4. Pagel JE, Qureshi AA, Young DM, Vlassoff LT. Comparison of four membrane filter methods for fecal coliform enumeration. Appl Environ Microbiol 1982; 43:787.
- AOAC. Official final action hydrophobic grid membrane filter method for detecting total coliforms, fecal coliforms and *E. coli* in foods. J Assoc Off Anal Chem 1985; 68:481.
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- 7. Andrews W. Official methods of analysis of AOAC International. 16<sup>th</sup> ed. Arlington, VA: AOAC International, 1995.
- 8. Eaton AD, Clesceri LS, Greenberg AE, Eds. Standard methods for the examination of water and wastewater. 19<sup>th</sup> ed. Washington, DC: APHA, 1999.
- 9. Sharpe AN. Enumeration of faecal coliforms in foods by the hydrophobic grid-membrane filter (HGMF) method, MFLP-55. Laval, Quebec: Polyscience Publications, 2001.

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