IFU

Instructions for Use

CRITERION™ M-FC AGAR BASE

Cat. no. C6270	CRITERION™ m-FC Agar Base	104gm
Cat. no. C6271	CRITERION™ m-FC Agar Base	500gm
Cat. no. C6272	CRITERION™ m-FC Agar Base	2kg
Cat. no. C6273	CRITERION™ m-FC Agar Base	10kg
Cat. no. C6274	CRITERION™ m-FC Agar Base	50kg

INTENDED USE

Hardy Diagnostics CRITERION™ m-FC Agar Base is recommended for thecultivation and enumeration of fecal coliforms in water samples usingthe membrane filtration technique at elevated temperatures. Rosolic acid may beadded to the media as a selective agent to further inhibit undesirable microorganisms during testing.

SUMMARY

Geldreich et al. formulated a medium to enumerate fecal coliforms (m-FC) using the membrane filter (MF) technique without prior enrichment.(1) Fecal coliforms, which are found in the gastro intestinal tracts and feces of warm-blooded animals, are differentiated from coliforms from environmental sources by their ability to grow at elevated temperatures: 44.5 +/- 0.2ºC.(2)

The m-FC method for detection of fecal coliforms can be used for monitoring all types of water. Because many coliforms occur naturally in environmental sources as well as through fecal contamination, *Escherichia coli* is recommended as the required indicator for freshwater testing and *E. coli* or enterococci as the required indicators for marine water testing since these microorganisms are known fecal contaminants.(5)

Hardy Diagnostics CRITERION™ m-FC Agar Base contains peptones as a source of carbon, nitrogen, vitamins and minerals. Yeast extract supplies B-complex vitamins that help stimulate bacterial growth. Lactose is a carbohydrate that can be fermented by fecal coliforms at elevated temperatures. Bile salts no. 3 inhibits the growth of undesirable gram-positive microbial flora and, when added, rosolic acid will further inhibit the growth of undesirable microorganisms. m-FC Agar Base contains agar as the solidifying agent. The differential indicator is aniline blue, which demonstrates the ability of fecal coliforms to ferment lactose to acid causing color change in the medium.

FORMULA*

Gram weight per liter:	52.0gm/L
Lactose	12.5gm
Tryptose	10.0gm

Sodium Chloride	5.0gm
Proteose Peptone No. 3	5.0gm
Yeast Extract	3.0gm
Bile Salts No. 3	1.5gm
Aniline Blue	0.1gm
1% Rosolic Acid**	10.0ml
Agar	15.0gm

****1% Rosolic Acid (when added)**	
Composition per 100ml, 0.2N Sodium Hydroxide	
Rosolic Acid	1.0gm

Final pH 7.4 +/- 0.2 at 25ºC.

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

STORAGE AND SHELF LIFE

Store the sealed bottle(s) containing dehydrated culture medium at 2-30ºC. Dehydrated culture medium is very hygroscopic. Keep lid tightly sealed. Protect dehydrated culture media from moisture and light. The dehydrated culture media should be discarded if it is not free-flowing or if the color has changed from its original beige with a slight blue tint.

Store the prepared culture media at 2-30ºC.

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended incubation times as stated below.

Refer to the document "Storage" for more information.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual universal blood precautions. Do not ingest, inhale, or allow to come into contact with skin.

This product is for laboratory use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "Guidelines for Isolation Precautions" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

METHOD OF PREPARATION FOR DEHYDRATED CULTURE MEDIA

1. Dissolve 52.0gm of the dehydrated culture media in 1 liter of distilled or deionized water. Stir to mix thoroughly.

2. Heat to boiling to dissolve completely. DO NOT OVERHEAT.

3. If desired, add 10ml of 1% rosolic acid solution prepared in 0.2N NaOH.

4. Continue heating for one minute. DO NOT AUTOCLAVE.

PROCEDURE

Sample Collection: Consult listed references for information on sample collection.[2,6-9]

Method of Use:

1. Prepare agar medium from dehydrated base according to the label directions outlined above.

2. Pour molten (cooled to 45-50ºC.) agar into tight-fitting petri plates and allow it to harden.

3. Filter duplicate water specimens through separate membrane filters.

4. Roll membrane filter used to collect the water sample onto the agar surface. Avoid the formation of air bubbles between the filter and agar surface.

5. Place plates into separate waterproof plastic bags and seal bags to prevent leakage.

6. Incubate plates, by immersion, in two separate waterbaths: one set at 35 +/- 2ºC. and the other at 44.5 +/- 0.2ºC.

7. Anchor plates below water surface to maintain critical temperature requirements and incubate for 24 +/- 2 hours. Place all inoculated plates in waterbath within 30 minutes after filtration for best results. As an alternative, an appropriate and accurate solid heat sink or equivalent incubator may be used.

INTERPRETATION OF RESULTS

Colonies produced by fecal coliforms will appear as varying shades of blue-colored colonies on the membrane filter. Coliforms from other sources should be inhibited; however, if present they may appear as gray to cream-colored colonies.

Calculate fecal coliform densities as directed by listed references.[2,4-6]

LIMITATIONS

Even with the selective action of the elevated incubation temperature and, when added, rosolic acid, a few non-fecal coliforms may be observed. To eliminate the potential for *Klebsiella* contamination, it may be helpful to elevate the incubation temperature to 45 +/- 0.2ºC.

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification.

Some formulations may require a settling period before pH testing of the prepared medium. If the pH is tested immediately after preparation and is out of specification, retest the medium after 24 hours to obtain final pH results. Always take pH reading at room temperature.

Refer to the document "Limitations of Procedures and Warranty" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as autoclaves, incinerators, sodium hydroxide, rosolic acid, membrane filters, petri plates, plastic bags, incubators and waterbaths, etc., are not provided.

QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following microorganisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Escherichia coli ATCC® 25922	MF	24hr	44.5°C	Aerobic	Growth; blue colonies/media
Enterococcus faecalis ATCC® 19433	B	24hr	44.5°C	Aerobic	Inhibited

* Refer to the document "Inoculation Procedures for Media QC" for more information.

USER QUALITY CONTROL

Users of dehydrated culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificate of analysis (CofA) available from Hardy Diagnostics Certificate of Analysis website. In addition, refer to the following document "Finished Product Quality Control Procedures," for more information on QC or see the reference(s) for more specific information.

PHYSICAL APPEARANCE

CRITERION™ m-FC Agar Base should appear homogeneous, free-flowing, and beige with a slight blue tint. The prepared media, when supplemented with rosolic acid, should appear clear, slightly opalescent and cranberry in color.

REFERENCES

1. Geldreich E.E., H.F. Clark, C.B. Huff, and L.C. Best. 1965. *Fecal Coliform Organism Medium for the Membrane Filter Technique*. J. Am. Water Works Assoc.; 57:208-214.

2. American Public Health Association. 2005. *Standard Methods for the Examination of Water and Wastewater*, 21st ed. APHA, Washington, D.C.

3. Horwitz, W. 2000. *Official methods of analysis of AOAC International*, 17th ed. 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, OH.

5. Bordner, R.H., J.A. Winter and P.V. Scarpino. 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, OH.

6. Anderson, N.L., et al. *Cumitech 3B; Quality Systems in the Clinical Microbiology Laboratory*, Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.

7. Jorgensen., et al. *Manual of Clinical Microbiology,* American Society for Microbiology, Washington, D.C.

8. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology,* C.V. Mosby Company, St. Louis, MO.

9. Association of Official Analytical Chemists. *Official Methods of Analysis*[sm], AOAC, Washington, D.C.

ATCC is a registered trademark of the American Type Culture Collection.

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