IFU

HARDY DIAGNOSTICS
A Culture of Service™

Instructions for Use

KF STREPTOCOCCUS AGAR

Cat. no. G376	KF Streptococcus Agar, 15x100mm Plate, 19ml	10 plates/bag

INTENDED USE

Hardy Diagnostics KF Streptococcus Agar is intended for the selective isolation and enumeration of fecal streptococci (including *Enterococcus*) from water and food samples by direct culture or membrane filtration.[1,2]

This product is not intended to be used for the diagnosis of human disease.

SUMMARY

The natural habitat of fecal streptococci is the gastrointestinal tract of warm-blooded animals. The enterococcus subgroup of fecal streptococcus is a valuable bacteriological indicator for determining the extent of fecal contamination of recreational surface waters.

KF (Kenner Fecal) Streptococcus Agar is a selective medium developed by Kenner, Clark and Kabler for the isolation and enumeration of fecal streptococci in surface waters, food and other materials by direct culture or membrane filtration.[1,2,5,6] In the early 1960s, Kenner et al. compared the performance of this medium to other streptococcal media and showed that KF Streptococcus Agar yielded higher cell densities using both MPN tests and membrane filter counts; they recommended use of this medium for membrane filtration over the multiple tube technique.[5,7] As a result, current isolation and enumeration techniques for detecting fecal streptococci as an indicator of pollution using KF Streptococcus Agar are made according to the APHA recommended guidelines for the examination of surface waters and foods.[1,2]

Hardy Diagnostics KF Streptococcus Agar is based on Kenner, Clark and Kabler's formulation and includes peptone as a source of nitrogen, amino acids and carbon. Yeast extract provides a source of vitamins, amino acids and essential trace elements. Maltose and lactose provide carbon as an energy source. Sodium azide is a selective agent added to inhibit the growth of gram-negative microorganisms. Bromcresol purple dye acts as an indicator. Triphenyl Tetrazolium Chloride (TTC) is a colorless redox agent added to differentiate colonies on agar or membrane filters. When irreversibly reduced to formazan by actively growing cells, TTC forms a cellular insoluble red pigment that can be exploited for more efficient enumeration.[3,4]

FORMULA

Ingredients per liter of deionized water:*

Ingredient	Amount
Maltose	20.0gm
Proteose Peptone No. 3	10.0gm
Yeast Extract	10.0gm
Sodium Glycerophosphate	10.0gm

Sodium Chloride	5.0gm
Lactose	1.0gm
Sodium Azide	0.4gm
Bromcresol Purple	15.0mg
1% Triphenyl Tetrazolium Chloride	10.0ml
Agar	20.0gm

Final pH 7.2 +/- 0.2 at 25ºC.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8ºC. away from direct light. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

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.

PROCEDURE

Direct Plating Procedure:

1. Inoculate plates using a 10^{-3} or greater dilution using the spread plate technique to obtain isolated colonies.

2. Incubate plates inverted at 35 +/- 2ºC. for 46 to 48 hours.

3. Count all red to pink colonies and report as the number of fecal enterococci calculated per sample.

Membrane Filter Procedure:

1. Filter a suitable volume of sample through a sterile membrane filter.

2. Place the inoculated membrane on the agar surface, inoculum side up, using a rolling motion to ensure proper contact with the surface and to avoid entrapment of air bubbles.

3. Incubate plates inverted at 35 +/- 2ºC. for 46 to 48 hours.

4. Count all red to pink colonies and report as the number of fecal enterococci calculated per volume of sample.

INTERPRETATION OF RESULTS

Fecal enterococci will appear as red to pink centered colonies. Using a dissecting microscope with 15X magnification or a colony counter can aid in determining colony counts.

LIMITATIONS

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

Due to varying nutritional requirements, some strains may grow poorly or fail to grow at all on this medium.

Some strains of *Streptococcus bovis* and *Streptococcus equinus* may be inhibited by azide.

The growth of orange, yellow, white or other colored colonies should not be counted.

Tropical marine water samples should be incubated anaerobically to avoid excessive numbers of false-positive presumptive counts for enterococci.

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

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, swabs, applicator sticks, other culture media, incinerators, membrane filters, and incubators, etc., as well as serological and biochemical reagents, 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	
Enterococcus faecalis ATCC® 29212	A	46-48hr	35°C	Aerobic	Growth; colonies with red to pink centers
Escherichia coli ATCC® 25922	B	48hr	35°C	Aerobic	Partial to complete inhibition

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

USER QUALITY CONTROL

End users of commercially prepared 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. Also refer to the document "Finished Product Quality Control Procedures," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

REFERENCES

1. American Public Health Association. *Standard Methods for the Examination of Water and Wastewater,* APHA, Washington, D.C.

2. APHA Technical Committee on Microbiological Methods for Foods. *Compendium of Methods for the Microbiological Examination of Foods,* APHA, Washington, D.C.

3. Gershman, M., D.C. O'Meara and H.L. Chute. 1959. Use of Tetrazolium Salt for an Easily Discernible Sulfide-Motility Reaction. *J. Bact.;* 78(5):739-740.

4. Kelly, A.T. and M. Fulton. 1953. Use of Triphenyl Tetrazolium Chloride in Motility Test Medium. *Am. J. Clin. Pathol.;* 23:512.

5. Kenner, B.A., H.F. Clark and P.W. Kabler. 1960. Quantification of Streptococci in Faeces. *Am. J. Publ. Health;* 50:1553-1559.

6. Kenner, B.A., H.F. Clark and P.W. Kabler. 1961. Cultivation and Enumeration of Streptococci in Surface Waters. *Appl. Microbiol.;* 9:15-20.

7. Slanetz, L.W. and C.H. Bartley. 1964. Detection and Sanitary Significance of Fecal Streptococci in Water. *Am. J. Pub. Health Nat. Health;* 54(4):609-614.

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