IFU

Instructions for Use

CRITERION™ SUGAR FREE AGAR

Cat. no. C9341	CRITERION™ Sugar Free Agar	500g

INTENDED USE

Hardy Diagnostics CRITERION™ Sugar Free Agar is recommended for the enumeration of microbial contaminants in butter or other processed dairy products as described by the International Dairy Federation.[1]

Dehydrated culture media is a raw material not intended for use in the diagnosis of human disease. For implementation, this product requires additional processing and supplementation of ingredients before use.

SUMMARY

Sugar Free Agar was first developed by Ritter and Eschmann and further described by Thomas and Mossel.[2-4] The formulation is described by the International Dairy Federation for the enumeration of psychrotrophic and mesophilic gram-negative rods in butter and other processed dairy products.[1] Dairy products, like butter, may become contaminated during the manufacturing process. Though pasteurization normally destroys contaminating microorganisms in the final product, it may be useful for dairy manufacturers to routinely monitor the manufacturing process at various stages to trace the source of contamination.

Gram-negative rods commonly found as contaminates in dairy products are able to deaminate proteins as a source of carbon, while other organisms such as enterococci are inhibited by the lack of a carbohydrate source.[5] Hardy Diagnostics CRITERION™ Sugar Free Agar contains gelatin and tryptone peptones, which can be broken down by target strains and utilized as a carbon source for cellular metabolism. Sodium chloride is added to help cells maintain osmotic equilibrium. Agar is the solidifying agent. The medium conforms to the formulation described in the International Dairy Federation (I.D.F.).[1]

FORMULA*

Gram weight per liter:	34.0g/L
Gelatin Peptone	7.5g
Tryptone	7.5g
Sodium Chloride	5.0g
Agar	14.0g

Final pH 7.6 +/- 0.2 at 25ºC.

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

STORAGE AND SHELF LIFE

Store the sealed bottle(s) that contain dehydrated culture medium at 2-30ºC. Dehydrated culture medium is very hygroscopic and will clump when exposed to moisture and air. Keep lid tightly sealed. Protect dehydrated culture media from moisture and light. Dehydrated culture media should be discarded if clumped, if the media is not free-flowing or if the color has changed from its original light beige.

Store the prepared culture media at 2-8ºC and do not remove the container desiccant, if applicable.

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. Suspend 34.2g of the dehydrated culture media in one liter of distilled or deionized water. Stir to mix thoroughly.

2. Boil for one minute to completely dissolve**.**

3. Sterilize in the autoclave at 118ºC. for 12 minutes.

4. Cool to 45-50ºC and dispense as desired.

Note: The shelf life of in-house prepared media from dehydrated culture media is dependent upon preparation methods, container quality, equipment, storage conditions, and batch testing criteria and must be validated by the end user. Refer to *USP Microbiological Best Laboratory Practices <1117>* for more information on validation procedures.[(1)]

PROCEDURE

Refer to the listed reference for the preparation of samples for enumeration procedures.[(1)]

INTERPRETATION OF RESULTS

Refer to the listed reference for interpretation of results.[(1)] It is recommend that biochemical testing be completed on isolates for further identification.

LIMITATIONS

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.

Accurate counting may be difficult with molds or spreading colonies.

Rare, fastidious microorganisms may not grow on selective media formulations.

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, incubators, tubes, bottles, petri dishes, 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	
Staphylococcus aureus ATCC® 6538	J	18-48hr	35°C	Aerobic	Growth
Pseudomonas aeruginosa ATCC® 9027	J	18-48hr	35°C	Aerobic	Growth
Bacillus subtilis ATCC® 6633	J	18-48hr	35°C	Aerobic	Growth
Escherichia coli ATCC® 8739	J	18-48hr	35°C	Aerobic	Growth
Candida albicans ATCC® 10231	J	5-7days	20-25°C	Aerobic	Growth
Aspergillus brasiliensis ATCC® 16404	J	5-7days	20-25°C	Aerobic	Growth
Bifidobacterium breve ATCC® 15700	B	18-24hr	35°C	Aerobic	Inhibited
Lactobacillus acidophilus ATCC® 4356	B	18-24hr	35°C	CO_2**	Inhibited

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

** Atmosphere of incubation is enriched with 5-10% CO_2.

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™ Sugar Free Agar powder should appear homogeneous, free-flowing, and light beige in color. The prepared medium should appear light to medium amber to tan in color.

REFERENCES

1. International Dairy Federation. 1964. International standard count of contaminating organisms in butter. *International Standards* FIL-IDF 30.

2. Ritter P. and K.H. Eschmann. 1966. The bacteriological testing and assessment of table butter. *Alimenta*. 2:43-45.

3.Thomas S. B. 1969. Methods of assessing the psychrotrophic bacterial content of milk. *J. Appl. Bacteriol.* 3(3)2: 269

4. Mossel, D.A.A, B.Krol, and P.C. Moerman. 1972. *Alimenta*. 11(2):51-60.

5. American Public Health Association. *Standard Methods for the Examination of Dairy Products.* APHA, Washington, D.C.

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Distribution Centers:
California · Washington · Utah · Arizona · Texas · Ohio · New York · Florida · North Carolina

The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.

HDQA 2207B [D]