IFU

Instructions for Use

CRITERION™ SKIM MILK AGAR

Cat. no. C7930	CRITERION™ Skim Milk Agar	142gm
Cat. no. C7931	CRITERION™ Skim Milk Agar	500gm
Cat. no. C7932	CRITERION™ Skim Milk Agar	2kg
Cat. no. C7933	CRITERION™ Skim Milk Agar	10kg
Cat. no. C7934	CRITERION™ Skim Milk Agar	50kg

INTENDED USE

Hardy Diagnostics CRITERION™ Skim Milk Agar is used for the cultivation and differentiation of microorganisms based on proteolytic activity.

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

SUMMARY

Skim Milk Agar is commonly used to demonstrate proteolysis by organisms capable of hydrolyzing casein. Proteolytic bacteria use the enzyme caseinase to hydrolyze casein and form soluble nitrogenous compounds displayed as a clear zone around colonies. This clearing is much more pronounced on agar containing milk if the bacteria are able to produce acid from fermentable carbohydrates in the medium. Moreover, the hydrolysis of casein is often used to evaluate proteolytic activity of psychrotrophic microorganisms of significance to food, water, and dairy industries.[1,3,5]

Psychrotrophs, such as *Pseudomonas aeruginosa*, are strongly proteolytic and often responsible for spoilage of meat and dairy foods; this spoilage can result in a stale, bitter or rancid taste and smell.[3,4] It is generally known that *Pseudomonas* species are most often responsible for the spoilage of fish.[5] In addition, pseudomonads are often isolated from large bodies of water, and some species have been linked to eye, ear, and skin infections from recreational water use. Thus, pseudomonads may serve as an indicator of recreational water quality. *P. aeruginosa* is also commonly found in drinking water and is known to be very resistant to ozonation and chemical disinfection in swimming pools.[2]

Many methods have been used to enumerate *P. aeruginosa* from water, but the most-probable-number (MPN) method results in satisfactory recovery of the organism.[1] However, this method is not suitable for large-volume water testing and lacks precision. The membrane filtration (MF) technique eliminates these deficiencies.

CRITERION™ Skim Milk Agar is an improved formulation from standard skim milk formulas and provides greater sensitivity.[11] CRITERION™ Skim Milk Agar contains casein peptone and glucose to support growth. Yeast extract is added as a vitamin source. The medium is a modification of Brown and Foster's formulation and can be used as a differential and confirmatory test for the identification of *P. aeruginosa* in water.[1] *P. aeruginosa* hydrolyzes casein as indicated by a zone of clearing around the colonies. There may also be a yellow to green pigment diffused into the

medium.

FORMULA*

Ingredients per liter of deionized water:*

Gram weight per liter:	71.0gm/L
Dry Milk, Instant Nonfat	50.0gm
Pancreatic Digest of Casein	5.0gm
Yeast Extract	2.5gm
Glucose	1.0gm
Agar	12.5gm

Final pH 6.8 +/- 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 white to off white.

Store the prepared culture media at 2-8ºC.

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 71.0gm of the dehydrated culture medium in 1 liter of deionized water. Stir to mix thoroughly.

2. Gently heat to dissolve completely.

3. This is a heat sensitive product. Based on individual autoclaves, time or temperature adjustments may be necessary. Sterilize in the autoclave at:

- 118ºC. for 12 minutes.
- 121ºC. for 10 minutes.

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

PROCEDURE AND INTERPRETATION OF RESULTS

For information on procedures and interpretation of results, consult listed references.[1-5]

For the presumptive detection of *P. aeruginosa* from recreational water, a 200-500ml sample of water is filtered through a sterile membrane filter. The membrane is then placed on a plate of mPA Agar (Cat. no. G133). Inoculated plates are inverted and incubated at 41.5ºC. for 72 hours. Colonies are isolated from mPA Agar and subcultured using a direct inoculum to Skim Milk Agar by placing a single streak 2 to 4cm long down the center of the plate. Incubate Skim Milk Agar plates at 35ºC. for 24 to 48 hours and observe for characteristic clearing and pigment formation.[1]

For food and dairy testing of *P. aeruginosa* using CRITERION™ Skim Milk Agar, see listed references.[3,5]

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.

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, and incubators, 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	
Pseudomonas aeruginosa ATCC® 27853	*	24-48hr	35°C	Aerobic	Growth; clear zone surrounding the colonies may have a yellowish to green pigment
Escherichia coli					

ATCC® 25922	*	24-48hr	35°C	Aerobic	Growth; no clear zone

* 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™ Skim Milk Agar powder should appear homogeneous, free-flowing, and white to off-white in color. The prepared media should appear opaque, and white in color.

REFERENCES

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

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4. Hui, Y.H., Pierson, M.D., Gorham, J.R. 2001. *Foodborne Disease Handbook*.& 2nd ed. Marcel Dekker, Inc., New York, NY.

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

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. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I, II & III. American Society for Microbiology, Washington, D.C.

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11. Martley, F.G., et al. 1969. *J. Appl. Bact.*; 33:363-370.

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

HDQA 2207B [D]