SKIM MILK AGAR

Cat. no. G138	Skim Milk Agar, 15x60mm Plate, 11ml	10 plates/bag

INTENDED USE

Hardy Diagnostics Skim Milk Agar is used to determine proteolysis by microorganisms and is recommended for the cultivation and confirmation of *Pseudomonas aeruginosa* in the testing of recreational waters.[1,2] This media is also useful for the detection of *Pseudomonas aeruginosa* in the food and dairy industry.[3,5]

SUMMARY

Many different methods have been used to enumerate *Pseudomonas aeruginosa* from water samples. The most-probable-number (MPN) procedures result in satisfactory recovery of *Pseudomonas aeruginosa* , but are not suitable for large-volume water testing and lack precision. The membrane filter (MF) techniques eliminate these deficiencies. Skim Milk Agar acts as a differential and confirmatory agar for the identification of *Pseudomaonas aeruginosa* in water.[1]

Pseudomonas species are possibly the organism most often isolated from large bodies of water. Some *Pseudomonas* species have been linked to eye, ear and skin infections after exposure to recreational bodies of water and thus may serve as an indicator of recreational water quality. *Pseudomonas aeruginosa* is commonly found in drinking water. *Pseudomonas aeruginosa* has been found to be very resistant to ozonation processes and chemical disinfection in swimming pools and thus underscores its importance.[2]

Psychrotrophic bacteria, such as *Pseudomonas aeruginosa* , are strongly proteolytic and are often responsible for the spoilage of both meat and dairy foods.[3,4] This spoilage can result in a stale, bitter or rancid taste and smell.[3] It is generally known that *Pseudomonas* species are the organisms most often responsible for the spoilage of fish.[5]

The hydrolysis of casein (a primary milk protein) is often used to evaluate proteolytic activity of organisms. The enzyme caseinase hydrolyzes the protein casein. Hardy Diagnostics Skim Milk Agar is an improved formulation from standard skim milk formulas, demonstrating a greater sensitivity.[11] It contains casein and glucose as carbon sources for growth promotion. Yeast extract is added to the medium as a vitamin source. *Pseudomonas aeruginosa* hydrolyzes casein and is indicated by a zone of clearing around the colonies. There may also be a yellow to green pigment diffused in the media.[1]

FORMULA

Ingredients per liter of deionized water:*

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 +/- 0.3 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

For the presumptive detection of *Pseudomonas aeruginosa* from a recreational water, a 200-500ml sample of water is filtered through a sterile membrane filter. The membrane is then placed on a poured plate of Modified m PA (Cat. no. G133). Inoculated plates are inverted and incubated at 41.5ºC. for 72 hours. Colonies are isolated from m PA and sub cultured onto the Skim Milk Agar (Cat. no. G138) making a single streak 2 to 4cm long and incubated at 35ºC. for 24-48 hours as a confirmatory test.[1]

For food and dairy testing of *Pseudomonas aeruginosa* using Skim Milk Agar (Cat. no. G138), see listed references below.[3,5]

INTERPRETATION OF RESULTS

A positive reaction is indicated by a clearing in the media surrounding the colonies. *Pseudomonas aeruginosa* will hydrolyze casein and may produce a yellow to green diffusible pigment.[1]

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.

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, other culture media, swabs, applicator sticks, incinerators, 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	
Pseudomonas aeruginosa ATCC[®] 27853	*	24-48hr	35°C	Aerobic	Clear zone surrounding the colonies, may have a yellowish to green pigment
Escherichia coli ATCC[®] 25922	*	24-48hr	35°C	Aerobic	No clear zone

* 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.

PHYSICAL APPEARANCE

Skim Milk Agar should appear opaque, and white in color.

Pseudomonas aeruginosa (ATCC® 27853) growing on Skim Milk Agar (Cat. no. G138). Incubated aerobically for 24 hours at 35ºC.

Escherichia coli (ATCC® 25922) growing on Skim Milk Agar (Cat. no. G138). Incubated aerobically for 24 hours at 35ºC.

REFERENCES

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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.

10. MacFaddin, J.F. 1985. *Media for Isolation, Cultivation, Identification, Maintenance of Bacteria* , Vol. I. Williams & Wilkins, Baltimore, MD.

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

HDQA 2207D [D]