IFU

Instructions for Use

M-PA AGAR

Cat. no. G133	m-PA Agar, 15x60mm Plate, 11ml	10 plates/bag

INTENDED USE

Hardy Diagnostics m-PA Agar is recommended for the cultivation and enumeration of *Pseudomonas aeruginosa* in water by membrane filtration.

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

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 *P. aeruginosa*, but are not suitable for large-volume water testing and lack precision. The membrane filter (MF) techniques eliminate these deficiencies.

Levin and Cabelli formulated m-PA Agar as a selective membrane filter medium for *P. aeruginosa*.[1] m-PA Agar contains kanamycin, nalidixic acid, sulfapyridine and cycloheximide to make it moderately selective. This formulation is found in the *Standard Methods for the Examination of Water and Wastewater*.[4]

FORMULA

Ingredients per liter of deionized water:*

Sodium Thiosulfate	6.8gm
L-Lysine Hydrochloride	5.0gm
Sodium Chloride	5.0gm
Xylose	2.5gm
Yeast Extract	2.0gm
Sucrose	1.25gm
Lactose	1.25gm
Ferric Ammonium Citrate	800.0mg
Sulfapyridine	176.0mg
Cycloheximide	150.0mg
Phenol Red	80.0mg
Nalidixic Acid	37.0mg

Kanamycin	8.5mg
Agar	15.0gm

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

1. Filter water sample through a sterile 47mm, 0.45um gridded filter.

2. Place the membrane filter on the surface of prepared m-PA Agar plates. Avoid trapping bubbles between the agar and filter.

3. Invert plates and incubate for 72 hours at 41.5ºC.

Consult the standard method for additional information regarding the m-PA membrane filter technique.[4]

INTERPRETATION OF RESULTS

0.8-2.2mm colonies that are flat in appearance with light outer rims and brownish to greenish-black centers are indicative of *Pseudomonas aeruginosa*. All such colonies should be counted and reported as total count per volume of water sampled. Dilution factors must taken into account.

Milk Agar may be used to confirm counts of typical and atypical colonies. Consult standard methods for more information.[4]

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	MF	18-24hr	35°C	Aerobic	Growth
Escherichia coli ATCC® 25922	B	18-24hr	35°C	Aerobic	Partial to complete inhibition
Proteus mirabilis ATCC® 12453	B	18-24hr	35°C	Aerobic	Partial to complete inhibition, no swarming

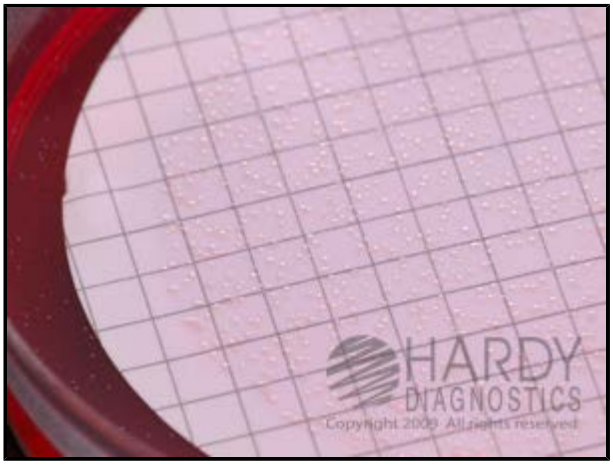
* Refer to the document "Inoculation Procedures for Media QC" for more information.

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

m-PA Agar should appear clear, and medium orange red to rose red in color.



Pseudomonas aeruginosa (ATCC® 27853) filtered through a white membrane (Cat. no. A045H047A) and growing on m-PA Agar (Cat. no. G133). Incubated aerobically for 24 hours at 35ºC.

Pseudomonas aeruginosa (ATCC® 27853) growing on m-PA Agar (Cat. no. G133). Incubated aerobically for 24 hours at 35ºC.

Uninoculated plate of m-PA Agar (Cat. no. G133).

REFERENCES

1. Levin and Cabelli. 1972. *Appl. Microbiol.*; 24:864.

2. Carson, Peterson, Favero, Doto, Collins, and Lecin. 1975. *Appl. Microbiol.*; 30:935.

3. Dutka and Kwan. 1977. *Appl. Environ. Microbiol.*; 33:240.

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

5. Brodsky and Ciebin. 1978. *Appl. Environ. Microbiol.*; 36:36.

6. Estevez. 1984. Bacteriologic plate media: review of mechanisms of action. *Lab. Med.*; 15:258.

IFU-10858[A]

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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 2207D [D]