PRODUCT SPECIFICATION SHEET

Pseudomonas Agar (For Pyocyanin) (DM219)

Intended Use

Pseudomonas Agar (For Pyocyanin) (DM219) is recommended for detection of pyocyanin production by Pseudomonas species

Product Summary and Explanation

Pseudomonas aeruginosa is one of the most commonly isolated pathogens, and is widely distributed in soil, water and foods. It is the most frequently isolated non-fermentative bacillus in clinical specimens, infusion fluids, disinfectants and cosmetics. The organism causes disease in humans; e.g., ocular infections, burn wound infections and respiratory tract infections.[1, 2] This organism is a significant cause of burn and nosocomial infections.[3] The ability of *Pseudomonas aeruginosa* to destroy tissue may be related to the production of various extracellular enzymes.[2]

Pseudomonas aeruginosa produces a number of water-soluble pigments. Most of the strains of *Pseudomonas aeruginosa* produce pyocyanin, a blue, water- and chloroform-soluble, non-fluorescent pigment that diffuses into the surrounding medium. *Pseudomonas aeruginosa* is the only *Pseudomonas* species known to produce this pigment. (However, certain strains are apyocyanogenic).[4] Some strains of *Pseudomonas aeruginosa* produce other pigments, such as the brown-black pyomelanin, the red pyorubin or the yellow pyoverdin. Pyoverdin is a water soluble fluorescent pigment often produced by *Pseudomonas aeruginosa* and other pseudomonads isolated from humans. When pyoverdin combines with the blue water-soluble pigment pyocyanin, the bright green color characteristic of *Pseudomonas aeruginosa* is created. The presence of these pigments can, however, mask the production of pyocyanin.

Pseudomonas Agar is based on the formulation described by King et al[5] and as recommended in U.S. Pharmacopoeia[6] for detecting pyocyanin. This medium enhances the elaboration of pyocyanin but inhibits the formation of fluorescein pigment. Both pigments diffuse from *Pseudomonas* colonies into the medium when they grow. Pyocyanin elaborated on Pseudomonas P Agar is a blue color.

Principles of the Procedure

Pseudomonas Agar (For Pyocyanin) contains peptic digest of animal tissue which provides amino acids and other essential nitrogenous substances. Potassium sulphate and magnesium chloride enhances the pyocyanin production and suppresses the fluorescein production. Glycerol is supplemented as a source of carbon.

Formula / Liter

Ingredients	Gms / Liter
Peptic digest of animal tissue	20.00
Potassium sulphate	10.00
Magnesium chloride	1.40
Agar	15.00
Final pH: 7.0 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 46.40 grams of the medium in one liter of distilled water containing 10 ml glycerol.
2. Heat to boiling, to dissolve the medium completely.
3. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.

Quality Control Specifications

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Dehydrated Appearance	Cream to yellow homogeneous free flowing powder
Prepared Medium	Yellow coloured clear to slightly opalescent gel forms in Petri plates
Reaction of 4.64% w/v aqueous solution containing 1% v/v glycerol	pH 7.0 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 1.5% Agar gel

Expected Cultural Response: Cultural characteristics observed after an incubation at 35-37°C for 18-84 hours.

Sr. No.	Organisms	Results to be achieved				
		Inoculum (CFU)	Growth	Observed Lot value (CFU)	Recovery	Colour of Medium
1.	*Pseudomonas aeruginosa ATCC 9027*	50-100	good-luxuriant	25 -100	>=50 %	blue-green
2.	*Pseudomonas aeruginosa ATCC 27853*	50-100	good-luxuriant	25 -100	>=50 %	blue-green

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Obtain inoculum from a pure 18–24 hours culture of organism to be tested.
2. Inoculate plates or agar slants by streaking the surface.
3. Incubate at 35-37°C for 18–24 hours.
4. If the isolate fails to grow or grows slowly, reincubate at 25-30°C for 1-2 days and observe for growth and pigment production.

Results

1. A positive result is indicated by a blue pigment diffusing into the agar.
2. Pseudomonas Agar (For Pyocyanin), a blue to blue-green pigment seen in the colonies and surrounding medium.
3. Confirm the presence of pyocyanin by adding several drops of chloroform and observe for a blue color in the chloroform. (Pyocyanin is more soluble in chloroform than in water).

Storage

Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Occasionally, a *Pseudomonas* culture is encountered that will produce small amounts of pigment in the medium, indicated as a blue-green color on Pseudomonas Agar (For Pyocyanin). If a blue-green color occurs confirmation of the presence of pyocyanin can be made by extraction with chloroform ($CHCl_3$).
2. The formation of nonpigmented colonies does not completely rule-out *a Pseudomonas aeruginosa* isolate.
3. A pyocyanin-producing *Pseudomonas* strain will usually also produce fluorescin. It must, therefore, be differentiated from other simple fluorescent pseudomonads by other means. Temperature can be a determining factor as most other fl uorescent strains will not grow at 35°C. Rather, they grow at 25-30°C.

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Packaging
Product Name : Pseudomonas Agar (For Pyocyanin)
Product Code : DM219
Available Pack sizes : 100gm/ 500gm

References

1. Kiska and Gilligan. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
2. Baron, E. J., L. R. Peterson, and S. M. Finegold. 1994. Nonfermentative gram-negative bacilli and coccobacilli, p. 386-405. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc. St. Louis, MO.
3. Gilligan, P. H. 1995. *Pseudomonas* and *Burkholderia*, p. 509-519. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (eds.)., Manual of clinical microbiology, 6th ed. American Society of Microbiology, Washington, D.C.
4. Forbes, Sahm and Weissfeld. 2007. Bailey & Scott's diagnostic microbiology, 12th ed. Mosby, Inc., St. Louis, Mo.
5. King, Ward and Raney, 1954, J.Lab. and Clin. Med., 44:301
6. The United States Pharmacopoeia, 2008, The United States Pharmacopoeial Convention, Rockville, MD.

Further Information

For further information please contact your local MICROMASTER Representative.

MICROMASTER LABORATORIES PRIVATE LIMITED
Unit 38/39, Kalpataru Industrial Estate,
Off G.B. Road, Near 'R-Mall' , Thane (W) – 400607. M.S. INDIA.
Ph: +91-22-25895505, 4760, 4681. Cell: 9320126789.
Email: micromaster@micromasterlab.com
sales@micromasterlab.com

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01.01.2018	01.01.2018	01.01.2018
Microbiologist	**Head Quality Control**	**Head Quality Assurance**