PRODUCT SPECIFICATION SHEET

Pseudomonas Agar Medium For Detection of Fluorescein (DM218I)

Intended Use
Pseudomonas Agar Medium For Detection of Fluorescein (DM218I) is recommended for detection of fluorescein production by *Pseudomonas* species in compliance with IP.

Product Summary and Explanation
Pseudomonas aeruginosa is one of the most commonly isolated pathogens, and is the most frequently isolated non-fermentative bacillus in clinical specimens.[1] This organism is a significant cause of burn and nosocomial infections[2] The ability of *Pseudomonas aeruginosa* to destroy tissue may be related to the production of various extracellular enzymes.[1] They are also common contaminant of pharmaceutical and cosmetics related preparations. *Pseudomonas* produces phenazine pigments like Pyocyanin- blue green redox-active secondary metabolite pigment, pyorubin-rust brown pigment, -oxyphenzine- a breakdown product of Pyocyanin, pyoverdin-a water soluble yellow green pigments also known as fluorescein. Pseudomonas Agar (For Fluorescein) is based on the formula described by King et al[3] and as modified in the Indian Pharmacopeia[4] for the detection of fluorescein production a water soluble, chloroform insoluble fluorescent pigment by *Pseudomonas* species.[5] Both pigments diffuse from *Pseudomonas* colonies into the medium when they grow. This medium shows enhanced elaboration of fluorescein by *Pseudomonas* and inhibits the pyocyanin formation. A yellow fluorescent colouration is observed when the fluorescein pigment diffuses from the colonies of Pseudomonas into the agar. As some Pseudomonas strains produce small amounts of pyocyanin resulting in a yellow-green colouration.

Principles of the Procedure
Pseudomonas Agar Medium For Detection of Fluorescein contains peptic digest of animal tissue and pancreatic digest of casein which provides the essential nitrogenous nutrients, carbon, sulfur and trace elements for the growth of *Pseudomonas*. These nutrients are also favourable to fluroescein production. Peptone and phosphorous in the medium enhance the production of pyoverdin/fluorescein pigment. Dipotassium phosphate buffers the medium while magnesium sulphate provides necessary cations for the activation of fluorescein production. Glycerol is supplemented as a source of carbon.

Formula / Liter

Ingredients	Gms / Litre
Pancreatic digest of casein	10.00
Peptic digest of animal tissue	10.00
Anhydrous dibasic potassium phosphate	1.50
Magnesium sulphate, $7H_2O$	1.50
Agar	15.00
Final pH: 7.2 ± 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 37.23 grams of the medium in one litre purified/distilled water, containing 10 ml glycerin.
2. Heat to boiling to dissolve the medium completely.
3. Autoclave at 15 lbs pressure (121°C) for 15 minutes/as per validated cycle.

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Quality Control Specifications

Dehydrated Appearance	Cream to yellow homogeneous free flowing powder
Prepared Medium	Yellow coloured clear to slightly opalescent gel forms in Petri plates
Reaction of % Solution	Not Applicable
Gel Strength	Firm, comparable with 1.5% Agar gel

Growth Promotion Test
Growth Promotion is carried out in accordance with the harmonized method of IP. Cultural response was observed after an incubation at 33-37°C for not less than 3 days. Recovery rate is considered as 100% for bacteria growth on Soyabean Casein Digest Agar.

Expected Cultural Response: Cultural characteristics observed after incubation at 33-37°C for 18-48 hours. Recovery rate is considered as 100% for bacteria growth on Soyabean Casein Digest Agar.

Sr. No.	Organisms	Results to be achieved					
		Inoculum (CFU)	Observed Lot value (CFU)	Recovery	Characteristic colonial morphology	Fluoresc-ence in UV light	Oxidase
	Test for *Pseudomonas aeruginosa*						
1.	*Pseudomonas aeruginosa ATCC 9027*	50-100	35 -100	>=70%	Generally colourless to yellowish	positive	positive
	Additional Microbiological Testing						
2.	*Pseudomonas aeruginosa ATCC 27853*	50-100	35 -100	>=70%	Generally colourless to yellowish	positive	positive

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

Test Procedure
Refer to appropriate references for standard test procedures.

Results
Examine colonies under ultraviolet light (Wood's lamp).[5] Take care when using UV illumination because it may have a bactericidal effect. Be sure there is good growth before placing the culture under UV light. A positive result is indicated by a light, bright green-yellow color diffusing into the agar with a fluorescent zone surrounding growth. Refer to appropriate references and standard test procedures for interpretation of results.

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.

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Limitations of the Procedure

1. Salt concentration exceeding 2% affects pigment production. UV illumination may be bactericidal, so make sure that there is good growth before placing culture under UV light.[5]
2. For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification.
3. Consult appropriate texts for detailed information and recommended procedures.

Packaging

Product Name : Pseudomonas Agar Medium For Detection of Fluorescein
Product Code : DM218I
Available Pack sizes : 100gm / 500gm

References

1. 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.
2. 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.
3. King, E. O., M. K. Ward, and E. E. Raney. 1954. Two simple media for the demonstration of pyocyanin and fluorescein. J. Lab. Clin. Med. 44:301.
4. Indian Pharmacopoeia, 2007, Government of India, Ministry of Health and Family Welfare, Publications and Information Directorate (CSIR), New Delhi.
5. MacFaddin, J. F. 1985. Media for isolation-cultivation-identification-maintenance medical bacteria, vol. 1. Williams & Wilkins, Baltimore, MD.

Further Information

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