



## Pseudomonas Agar Medium for Detection of Fluorescein

MAP120

(MU120/MM120)

### Intended Use:

Recommended for detection of fluorescein production by *Pseudomonas* species in accordance with USP & IP.

### Composition\*\*

Ingredients	g / L
Tryptone#	10.000
Peptone ##	10.000
Anhydrous dibasic potassium phosphate	1.500
Magnesium sulphate heptahydrate	1.500
Agar	15.000
pH after sterilization ( at 25°C)	7.2±0.2

\*\*Formula adjusted, standardized to suit performance parameters

# Pancreatic digest of casein ##- Peptic digest of animal tissue

### Directions

Suspend 37.23 grams of (the equivalent weight of dehydrated medium per litre) in 1000 ml purified/distilled water, containing 10 ml glycerin. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

### Principle And Interpretation

Pseudomonas Agar (For Fluorescein) is based on the formula described by King et al (1) and as modified in the U.S. Pharmacopeia, Indian pharmacopoeia(2,3) for the detection of fluorescein production a water soluble, chloroform insoluble fluorescent pigment by *Pseudomonas* species (4). *Pseudomonas* is ubiquitous in environment and is a common causative agent of burn, skin and nosocomial infections. They are also common contaminant of pharmaceutical and cosmetics related preparations. *Pseudomonas* strains are reported to produce phenazine pigments like Pyocyanin-blue green redox-active secondary metabolite pigment, pyorubin-rust brown pigment, -oxyphenazine- a breakdown product of Pyocyanin, pyoverdin-a water soluble yellow green pigments also known as fluorescein. This medium enhances the elaboration of fluorescein by *Pseudomonas* and inhibits the pyocyanin formation. The fluorescein pigment diffuses from the colonies of *Pseudomonas* into the agar and shows yellow fluorescent colouration. Some *Pseudomonas* strains produce small amounts of pyocyanin resulting in a yellow-green colouration. Tryptone provides the essential nitrogenous nutrients, carbon, sulfur and trace elements for the growth of *Pseudomonas*. These nutrients are also conducive to the production of fluoroescien. 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. 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 (1).

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### Specimen Collection and Handling:

For pharmaceutical samples follow appropriate techniques for handling specimens as per established guidelines (2,3). After use, contaminated materials must be sterilized by autoclaving before discarding.

### Warning and Precautions :

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

### Limitations :

1. UV illumination may be bactericidal, so make sure that there is good growth before placing culture under UV light (4).

### Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Please refer disclaimer Overleaf.

## Quality Control

### Appearance

Cream to yellow homogeneous free flowing powder

### Gelling

Firm, comparable with 1.5% Agar gel

### Colour and Clarity of prepared medium

Yellow coloured clear to slightly opalescent gel forms in Petri plates

### pH

7.00-7.40

### Growth Promotion Test

Growth Promotion is carried out in accordance with USP/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.

### 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.

Organism	Inoculum (CFU)	Observed Lot value (CFU)	Recovery	Characteristic colonial morphology	Fluorescence in UV light	Oxidase
<b>Test for <i>Pseudomonas aeruginosa</i></b>						
<sup>^</sup> <i>Pseudomonas paraaeruginosa</i> ATCC 9027 (00026*)	50 -100	35 -100	>=70 %	Generally colourless to yellowish	positive	positive
<b>Additional Microbiological Testing</b>						
<i>Pseudomonas aeruginosa</i> ATCC 27853 (00025*)	50 -100	35 -100	>=70 %	Generally colourless to yellowish	positive	positive

Key : \*Corresponding WDCM numbers. ^ Formerly known as *Pseudomonas aeruginosa*

## Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

## Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (5,6).

## Reference

- King, Ward and Raney, 1954, J.Lab. Clin. Med., 44 : 301.
- The United States Pharmacopoeia-National Formulary (USP-NF), 2022.
- Indian Pharmacopoeia, 2022, Indian Pharmacopoeia Commission, Ministry of Health and Family Welfare Government of India.
- MacFaddin J., 1985, Media for Isolation-Cultivation-Identification and Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.
- Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
- Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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### Disclaimer :

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