



Pseudomonas Agar (For Fluorescein)

M120

Pseudomonas Agar (For Fluorescein) is recommended for the detection of fluorescein production by *Pseudomonas* species.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	10.000
Proteose peptone	10.000
Dipotassium phosphate	1.500
Magnesium sulphate	1.500
Agar	15.000
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 38 grams in 1000 ml distilled water containing 10 ml glycerol. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. 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 (2) for the detection of fluorescein production a water soluble, chloroform insoluble fluorescent pigment by *Pseudomonas* species (3). The 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.

Casein enzymic hydrolysate and proteose peptone provide the essential nitrogenous nutrients, carbon, sulphur and trace elements for the growth of *Pseudomonas*. 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 (3).

A pyocyanin-producing *Pseudomonas* strain will usually also produce fluorescein. It must, therefore, be differentiated from other simple fluorescent pseudomonads by other means. Temperature can be a determining factor as most other fluorescent strains will not grow at 35°C. Rather, they grow at 25-30°C (3).

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

Reaction

Reaction of 3.8% w/v aqueous solution (containing 1% v/v glycerol) at 25°C. pH : 7.0±0.2

pH

6.80-7.20

Cultural Response

M120: Cultural characteristics observed with added 1% glycerol after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
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Cultural Response

<i>Pseudomonas aeruginosa</i> ATCC 17934	50-100	luxuriant	$\geq 70\%$	greenish yellow
<i>Pseudomonas aeruginosa</i> ATCC 27853	50-100	luxuriant	$\geq 70\%$	greenish yellow
<i>Pseudomonas aeruginosa</i> ATCC 9027	50-100	luxuriant	$\geq 70\%$	greenish yellow

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Reference

1. King, Ward and Raney, 1954, J. Lab. Clin. Med., 44 : 301.
2. The United States Pharmacopoeia, 2006, USP29/NF24, The United States Pharmacopoeial Convention, Rockville, MD.
3. MacFaddin J., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.

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