# Technical Information

## Pseudomonas Agar Medium for Detection of Pyocyanin

## Product Code: DM 1119U

**Application: -** Pseudomonas Agar Medium for detection of Pyocyanin is used for the detection of pyocyanin production by *Pseudomonas* species in accordance with United States Pharmacopoeia.

## Composition**

| Ingredients | Gms / Litre |
|---|---|
| Pancreatic digest of gelatin | 20.000 |
| Anhydrous potassium sulphate | 10.000 |
| Anhydrous magnesium chloride | 1.400 |
| Agar | 15.000 |
| pH after sterilization ( at 25°C) | 7.2±0.2 |

**Formula adjusted, standardized to suit performance parameters

## Principle & Interpretation

Pseudomonas Agar Medium for detection of Pyocyanin is based on the formulation described by King et al (1) and as used by U.S. Pharmacopoeia (2) for detecting pyocyanin, a water soluble pigment by *Pseudomonas* species from clinical specimens such as stools, wounds, and urine. (3). It is also recommended for microbial limit tests for pharmaceutical and other biological preparations by USP. *Pseudomonas* species are commonly isolated pathogen and is the significant causative agent of nosocomial, skin and burn infections. *Pseudomonas* strains are reported to produce 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. Pyocyanin is readily recovered in large quantities in sputum from patients with cystic fibrosis, an infection caused by *Pseudomonas* (4,5). This medium enhances the formation of Pyocyanin and/or pyorubin and reduces that of fluorescein.

Pancreatic digest of casein supplies essential nutrients for growth of Pseudomonas, while glycerol provides carbon and energy to the cell. The pyocyanin pigment diffuses from the colonies of *Pseudomonas* into the agar and shows blue colouration. Potassium sulphate and magnesium chloride enhances the pyocyanin production and suppresses the fluorescein production. Low content of phosphorous in the medium also aids in inhibiting the production of fluorescein. Some Pseudomonas strains produce small amounts of fluorescein resulting in a blue-green colouration.

Strains of *Pseudomonas aeruginosa* that may fail to produce pyocyanin are not detected in this medium. Production of other pigments may mask the presence of pyocyanin.

## Methodology

Suspend 46.4 grams of dehydrated powder media in 1000 ml distilled water containing 10 ml glycerin. Mix thoroughly & heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes or as per validated cycle.

## Quality Control

**Appearance**

Cream to yellow homogeneous free flowing powder.

**Gelling**

Firm comparable with 1.5% Agar gel.

**Colour and Clarity**

Yellow coloured clear to slightly opalescent gel forms in Petri plates.

**pH Range**

7.00-7.40

**Cultural Response**

Growth Promotion is carried out in accordance with the harmonized method of USP. 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**

| Organism | Inoculum (CFU) | Observed Lot value (CFU) | Recovery | Characteristic colonial morphology | Fluorescence in UV light | Oxidase |
|---|---|---|---|---|---|---|
| **Test for Pseudomonas aeruginosa** | | | | | | |
| *Pseudomonas aeruginosa ATCC 9027* | 50 -100 | 35 -100 | >=70 % | Generally greenish | Blue | positive |
| **Additional Microbiological Testing** | | | | | | |
| *Pseudomonas aeruginosa ATCC 27853* | 50 -100 | 35 -100 | >=70 % | Generally greenish | Blue | positive |

## Storage and Shelf Life

**Dried Media:** Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.
**Prepared Media**: 2-8° in sealable plastic bags for 2-5 days.

## Further Reading

1. King, Ward and Raney, 1954, J.Lab. and Clin. Med., 44:301
2. United States Pharmacopoeia, 2008, United States Pharmacopoeia Convention, Inc., Rockville, MD.
3. MacFaddin J., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams an Wilkins, Baltimore.
4. Daly J A, Boshard R, and Matsen J M, 1984, J Clin Microbiol. 19: 742
5. Lau GW, Hassett DJ, Ran H, Kong F., 2004. Trends Mol Med. 10:599

## Disclaimer :

- User must ensure suitability of the product(s) in their application prior to use.
- The product conform solely to the technical information provided in this booklet and to the best of knowledge research and development work carried at CDH is true and accurate
- Central Drug House Pvt. Ltd. reserves the right to make changes to specifications and information related to the products at any time.
- Products are not intended for human or animal diagnostic or therapeutic use but for laboratory, research or further manufacturing of diagnostic reagents extra.
- Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents. Do not use the products if it fails to meet specification for identity and performance parameters.