

Cetrimide HiCynth™ Agar Base

MCD024

Intended use

Recommended for the selective isolation of *Pseudomonas aeruginosa*.

Composition**

Ingredients	g/ L
HiCynth™ Peptone No.3*	20.000
Magnesium chloride	1.400
Potassium sulphate	10.000
Cetrimide	0.300
Agar	15.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

*Chemically defined peptone

Directions

Suspend 46.7 grams in 1000 ml purified / 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. Cool to 45-50°C. If desired, rehydrated contents of 1 vial of NA Selective Supplement (FD130) may be added aseptically to 1000 ml medium. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Pseudomonas aeruginosa grows well on all normal laboratory media but specific isolation of the organism, from environmental sites or from human, animal or plant sources, is best carried out on a medium, which contains a selective agent and also constituents to enhance pigment production. Most selective media depend upon the intrinsic resistance of the species to various antibacterial agents. Cetrimide inhibits the growth of many microorganisms whilst allowing *Pseudomonas aeruginosa* to develop typical colonies. Cetrimide is a quaternary ammonium salt, which acts as a cationic detergent that reduces surface tension in the point of contact and has precipitant, complexing and denaturing effects on bacterial membrane proteins. It exhibits inhibitory actions on a wide variety of microorganisms including *Pseudomonas* species other than *Pseudomonas aeruginosa*. King et al developed Medium A for the enhancement of pyocyanin production by *Pseudomonas* (1). Cetrimide Agar developed by Lowburry (2) is a modification of Tech Agar (Medium A) with addition of 0.1% cetrimide for selective isolation of *P. aeruginosa*. Later, due to the availability of the highly purified cetrimide, its concentration in the medium was decreased (3). The incubation was carried out at 37°C for a period of 18-24 hours (4).

Cetrimide HiCynth™ Agar Base is the modification prepared by replacing animal and vegetable based peptones with chemically based peptones to avoid BSE/TSE risks associated with animal peptones. *P.aeruginosa* can be identified due to their characteristic production of pyocyanin, a blue, water-soluble, non-fluorescent phenazine pigment coupled with their colonial morphology and the characteristic grape-like odor of amino aceto phenone(5). *P.aeruginosa* is the only species of *Pseudomonas* known to excrete pyocyanin. These media are therefore, important in the identification of *P. aeruginosa*. These media are used for the examination of cosmetics (6) and specimens (7,8) for the presence of *P. aeruginosa*, as well as for evaluating the efficacy of disinfectants against this organism (8). HiCynth™ PeptoneNo.3 provide necessary nutrients for *P. aeruginosa*. Sodium chloride maintains osmotic equilibrium in the medium. Magnesium chloride and potassium sulfate stimulates pyocyanin production (9). For the isolation of *P. aeruginosa*, plates of Cetrimide Agar should be inoculated from non-selective medium such as BHI HiCynth™ Broth (MCD210) or Tryptone Soya HiCynth™ Broth (MCD011). If the count is high, the test sample can be directly inoculated onto Cetrimide Agar. *P.aeruginosa* colonies may appear pigmented blue, blue-green or non-pigmented. Colonies exhibiting fluorescence at 250nm and a blue green pigmentation are considered as presumptive positive. *P. aeruginosa* may lose its fluorescence under UV if the cultures are left at room temperature for a short time.

Fluorescence reappears after the plates are re-incubated (4). Type of peptone used in the base may also affect pigment production (4,10). Certain strains of *P.aeruginosa* may not produce pyocyanin. Other species of *Pseudomonas* do not produce pyocyanin but fluoresce under UV light. Most non-*Pseudomonas* species are inhibited on Cetrimide Agar, and some species of *Pseudomonas* may also be inhibited. Some non-fermenters and some aerobic spore formers may exhibit a water-soluble tan to brown pigmentation on this medium. *Serratia* may exhibit pink pigmentation (3). Biochemical tests and serological procedures should be performed to confirm the findings.

Type of specimen

Water samples.

Specimen Collection and Handling:

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards(11). 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.This medium is a selective medium, some strains may show poor growth as cetrimide is highly toxic.
- 2.Further biochemical tests must be carried out for complete identification.

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.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Light amber coloured opalescent gel with a slight precipitate forms in Petri plates

Reaction

Reaction of 4.67% w/v aqueous solution containing 1% glycerol at 25°C . pH : 7.2±0.2

pH

7.00-7.40

Cultural Response

Cultural characteristics observed after incubation at 30-35 °C for 18-72 hours. Recovery rate is considered as 100% for bacteria growth on Soyabean Casein Digest HiCynth™ Agar.

Organism	Inoculum (CFU)	Growth	Recovery
^ <i>Pseudomonas</i> <i>paraeruginosa</i> ATCC 9027 (00026*)	50 -100	luxuriant	>=50 %
<i>Escherichia coli</i> ATCC 8739 (00012*)	>=10 ⁴	inhibited	0%

<i>Pseudomonas aeruginosa</i> ATCC 27853 (00025*)	50 -100	luxuriant	≥ 50 %
<i>Pseudomonas aeruginosa</i> ATCC 25668 (00114*)	50 -100	luxuriant	≥ 50 %
<i>Stenotrophomonas maltophilia</i> ATCC 13637	$\geq 10^4$	inhibited	0%
<i>Escherichia coli</i> ATCC 25922 (00013*)	$\geq 10^4$	inhibited	0%
<i>Staphylococcus aureus</i> subsp.aureus ATCC 6538 (00032*)	$\geq 10^4$	inhibited	0%
<i>Staphylococcus aureus</i> subsp.aureus ATCC 25923 (00034*)	$\geq 10^4$	inhibited	0 %
<i>Salmonella</i> Typhimurium ATCC 14028 (00031*)	$\geq 10^4$	inhibited	0%
<i>Proteus mirabilis</i> ATCC 29906 (00023*)	$\geq 10^4$	inhibited	0%

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 (12,13).

Reference

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