Technical Data Sheet

HEIMPLATE™ Cetrimide Agar

Ordering number: 1.46048.0020

90 mm settle plates is designed for selective isolation of Pseudomonas aerugi- nosa in non-sterile pharmaceutical products.

General

This medium complies with the specifications given by the harmonized methods of EP, USP, JP for Microbial Examination of Non-sterile Products: Tests for Specified Microorganisms.

Mode of Action

The use of cetrimide (cetyltrimethylammonium bromide) was recommended by Lowbury (1951) and other authors; this compound largely inhibits the growth of the accompanying microbial flora. According to Lowbury and Collins (1955), a concentration of 0.3 g/l inhibits the accompanying organisms satisfactorily and minimizes interference with the growth of Pseudomonas aeruginosa. The pigment production of Pseudomonas aeruginosa is not inhibited when grown on this medium.

Typical Composition (g/l)

Pancreatic Digest of Gelatin	20 g/l
$MgCl_2$	1.4 g/l
K_2SO_4	10 g/l
Cetrimide	0.3 g/l
Glycerol	10 ml/l
Agar	13.6 g/l

The appearance of the medium is slightly turbid with particles and colorless. The pH value is in the range of 7.0-7.4. The medium can be adjusted and/or supplemented according to the performance criteria required.

Application and Interpretation

Please check each agar plate before using it on sterility and pay attention to aseptic handling in order to avoid false positive results.

Pseudomonas aeruginosa is able to produce different pigments in different combinations. More than 90 % of all strains produce the bluish, non-fluorescent pyocyanin as well as the yellowish,

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fluorescent pyoverdin (= fluorescein). The production of the brownish to black pyomelanin and red pyorubin is very seldom.

According to the recommendations of the current EP and USP Cetrimide Agar is incubated for 18-72 h at 30-35 °C.

Note: Beside Pseudomonas aeruginosa also Pseudomonas putida and Pseudomonas fluorescens are able to grow on Cetrimide Agar at 30 °C to 35 °C, w hile Burkholderia cepacia and Stenotrophomonas maltophilia are inhibited. B. cepacia is able to grow at an incubation temperature of approx. 25 °C.

The growth of colonies indicates the possible presence of Pseudomonas aeruginosa. This confirmed by identification tests.

The product complies with the test if colonies are not present or if the confirmatory identification tests are negative. Pseudomonas aeruginosa is characterised by a sweetish smell like lime-tree blossoms or grapes (aminoacetophenone). Furthermore, the colonies will show ß-hemolysis on blood agar.

The most important Pseudomonads can be pre-differentiated following the characteristics in the table below.

Phenotypic differentiation of the most important *Pseudomonads* [1)]

Characteristic	***P. aeruginosa***	***P. fluoreszens***	***P. putida***	***P. stutzeri***
Oxidase	+	+	+	+
Growth at 41 °C	+	-	-	+/-
Pyoverdin [2)] („Fluorescein")	+	+	+	-
Pyocyanin [2)]	+	-	-	-
Gelatinase	+	+	-	-

[1)] Derived from Bergey`s Manual of Determinative Bacteriology (1994) 9th Edition. Williams & Wilkins (+ = 90 % or more of thestrains are positive; - = 90 % or more of the strains are negative)

[2)] Pyoverdin („Fluorescein") and Pyocyanin can be identified using Pseudomonas Agar P (article number 146024) and Pseudomonas Agar F (article number 146047)

Storage and Shelf Life

The product can be used for sampling until the expiry date if stored upright, protected from light and properly sealed at +15 °C to +25 °C.
Condensation can be prevented by avoiding quick temperature shifts and mechanical stress.

The testing procedures as described on the CoA can be started up to the expiry date printed on the label.

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Disposal

Please mind the respective regulations for the disposal of used culture medium (e.g. autoclave for 20 min at 121 °C, disinfect, incinerate etc.).

Quality Control

Control Strains	ATCC #	Inoculum CFU	Incubation	Expected Results
Pseudomonas aeruginosa	27853	10-100	16-72 h at 30-35 °C	Recover 50-200 %; good growth; greenish, medium-sized colonies; greening of nutrient medium; fluorescent under UV light
Pseudomonas aeruginosa	9027	10-100	16-72 h at 30-35 °C	Recovery 50-200 %; good growth; greenish, medium-sized colonies; greening of nutrient medium; fluorescent under UV light
Escherichia coli	8739	100-1,000	72-76 h at 30-35 °C	No growth

Please refer to the actual batch related Certificate of Analysis.

Pseudomonas aeruginosa ATCC 9027

Literature

Brown, V.I. and Lowbury E.J.L. (1965). Use of improved cetrimide agar medium and other culture methods for Pseudomonas aeruginosa. J. Clin. Pathol. 18: 752-756.

Buhlmann, X., Fischer, W.A. and Bruhn, J. (1961). Identification of a pyocyanogenic strains of

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Lowbury, E.J.L. (1951): Improved culture methods for the detection of Ps. pyocyanea. J. Clin. Pathol. 4: 66-72.

Lowbury, E.J.L. and Collins, A.G. (1955): The use of a new cetrimide product in a selective medium for

Pseudomonas pyocyanea. J. Clin. Pathol. 8: 47-48.

Thornley, M.J. (1960): The differentiation of Pseudomonas from other gram-negative bacteria on the basis of arginine metabolism. J. Appl. Bact. 23: 37-52.

United States Pharmacopoeia 38 NF 33 (2015): <62> Microbiological examination of non-sterile products: Tests for specified microorganisms.

Ordering Information

Product	Cat. No.	Pack size
HEIMPLATE™ Cetrimide Agar	1.46048.0020	20 x 90 mm
HEIMPLATE™ Pseudomonas Agar	1.46024.0020	20 x 90 mm

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