Technical Data Sheet

Cetrimide Agar
Pseudomonas Selective Agar Base
acc. harm. EP/USP/JP

Ordering number: 1.05284.0500 / 1.05284.5000

A modification of the medium proposed by Brown and Lowbury (1965) for the isolation and differentiation of *Pseudomonas aeruginosa* from various materials.

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 Ps. aeruginosa. The pigment production of *Pseudomonas aeruginosa* is not inhibited when grown on this medium.

Goto and Enomoto (1970) recommend the addition of 15 µg nalidixic acid/ml to improve the inhibition of the accompanying microbial flora.

Typical Composition

Peptone from Gelatin	20 g/l
$MgCl_2$	1.4 g/l
K_2SO_4	10 g/l
N-Cetyl-N,N,N-trimethylammoniumbromide (Cetrimide)	0.3 g/l
Agar-Agar	13.6 g/l
Glycerol	10 ml/l

Preparation

Suspend 45.3 g/l. Add 10 ml glycerol/l. Autoclave (15 min at 121 °C). Pour plates.

The appearance of the plates is turbid and light yellowish.

The pH value at 25 °C is in the range of 7.0-7.4.

Experimental Procedure and Evaluation

Inoculate by spreading the sample on the surface of the plates.

Incubation: *Pseudomonas aeruginosa* 18 h at 30-35 ℃, others 72 h.

Pseudomonas aeruginosa colonies produce the bluish, non-fluorescent pyocyanin as well as the yellow-green pigment pyoverdin that fluoresces under UV light. Further tests should be performed for further identification (Hugh and Leifson 1953, Kovacs 1956, Thornley 1960, Bühlmann et al. 1961 etc).

Note: Beside *Pseudomonas aeruginosa* also *Pseudomonas putida* and *Pseudomonas fluorescens* are able to grow on Cetrimide Agar at 30-35 ℃. 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 ℃	+	-	-	+/-
Pyoverdin („Fluorescein“)	+	+	+	-
Pyocyanin	+	-	-	-
Gelatinase	+	+	-	-

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

Storage

The product can be used for sampling until the expiry date if stored upright, protected from light and properly sealed at +15 ℃ to +25 ℃.

After first opening of the bottle the content can be used up to the expiry date when stored dry and tightly closed at +15 ℃ to +25 ℃.

Disposal

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

Quality Control

Control Strains	ATCC #	Inoculum CFU	Incubation	Expected Results
Pseudomonas aeruginosa	9027	10 - 100	18 h at 30-35 ℃	Recovery ≥ 50 %, yellow-green pigments
Pseudomonas aeruginosa	25668	10 - 100	18 h at 30-35 ℃	Recovery ≥ 50 %, yellow-green pigments
Pseudomonas aeruginosa	27853	10 - 100	18 h at 30-35 ℃	Recovery ≥ 50 %, yellow-green pigments
Escherichia coli	8739	> 10^4	72 h at 30-35 ℃	No growth
Proteus mirabilis	29906	> 10^4	72 h at 30-35 ℃	No growth
Salmonella Typhimurium	14028	> 10^4	72 h at 30-35 ℃	No growth
Staphylococcus aureus	6538	> 10^4	72 h at 30-35 ℃	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 *Pseudomonas aeruginosa*. J. Bact. **82**: 787-788.

DIN Deutsches Institut für Normung e.V. (1982): DIN 38411-8. Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung. Mikrobiologische Verfahren. Nachweis von *Pseudomonas aeruginosa*.

European Directorate for the Quality of Medicines and Healthcare. (2014): The European Pharmacopoeia. 8th Ed. Chapter 2.6.13 Microbiological examination of non-sterile products: Test for specified products. Strasbourg, France.

Goto, S. and Enomoto, S. (1970): Nalidixic acid cetrimide agar. A new selective plating medium for the selective isolation of *Pseudomonas aeruginosa*. Japan J. Microbiol. **14**: 65-72.

Hugh, R. and Leifson, E. (1953): The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various gramnegative bacteria. J. Bact. **66**: 24-26.

Japanese Ministry of Health, Labour and Welfare. (2011): The Japanese Pharmacopoeia. 16th Ed. Chapter 4.05 Microbial Limit Test II. Microbiological examination of non-sterile products: Test for specified products. Japanese Ministry of Health, Labour and Welfare. Tokyo, Japan.

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
Cetrimide Agar Pseudomonas Selective Agar Base	1.05284.0500	500 g
Glycerol (about 87 %)	1.04094.0500	500 ml
UV Lamp (366 nm)	1.13203.0001	1 piece

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