

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 ₂ SO 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 °C, 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 °C. The most important *Pseudomonads* can be pre-differentiated following the characteristics in the table below.

Phenotypic differentiation of the most important *Pseudomonads* ¹⁾

Characteristic	<i>P. aeruginosa</i>	<i>P. fluorescens</i>	<i>P. putida</i>	<i>P. stutzeri</i>
Oxidase	+	+	+	+
Growth at 41 °C	+	-	-	+/-
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 °C to +25 °C.

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

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.).



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Quality Control

Control Strains	ATCC #	Inoculum CFU	Incubation	Expected Results
<i>Pseudomonas aeruginosa</i>	9027	10 - 100	18 h at 30-35 °C	Recovery ≥ 50 %, yellow-green pigments
<i>Pseudomonas aeruginosa</i>	25668	10 - 100	18 h at 30-35 °C	Recovery ≥ 50 %, yellow-green pigments
<i>Pseudomonas aeruginosa</i>	27853	10 - 100	18 h at 30-35 °C	Recovery ≥ 50 %, yellow-green pigments
<i>Escherichia coli</i>	8739	> 10 ⁴	72 h at 30-35 °C	No growth
<i>Proteus mirabilis</i>	29906	> 10 ⁴	72 h at 30-35 °C	No growth
<i>Salmonella</i> Typhimurium	14028	> 10 ⁴	72 h at 30-35 °C	No growth
<i>Staphylococcus aureus</i>	6538	> 10 ⁴	72 h at 30-35 °C	No growth

Please refer to the actual batch related Certificate of Analysis.



Pseudomonas aeruginosa ATCC 9027



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Literature

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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.

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

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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 <i>Pseudomonas</i> 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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