

**Pseudomonas selective (Cetrimide) agar
(Ph.Eur., USP, JP)**

Code 84638.0500

Also known as

Cetrimide agar

Intended use

For the selective isolation and differentiation of *Pseudomonas aeruginosa*.

Formula * - Composition in g/L

Pancreatic digest of gelatin.....	20.0
Magnesium chloride.....	1.4
Potassium sulphate.....	10.0
Cetrimide.....	0.3
Agar.....	14.0

* Adjusted and/or supplemented as required to meet performance criteria

Final pH 7.2 ± 0.2 at 25 °C.

Instructions for preparation

Dissolve 45.7 g in 1 litre of purified water by bringing to the boil with frequent shaking. Add 10 ml of glycerol (Art.: 84730.0001). Sterilise in the autoclave at 121 °C for 15 minutes.

Principle of the method and general information

Pseudomonas selective (cetrimide) agar is a selective medium for the isolation and identification of *Pseudomonas* and is prepared according to a slight modification of the medium A described by King, Ward and Roney. *Pseudomonas selective (cetrimide) agar* corresponds to the Cetrimide agar recommended by European Pharmacopoeia. Pancreatic digest of gelatin provides nitrogen, carbon, essential amino acids, vitamins and minerals to support bacterial growth and stimulates the pigments production. Glycerol provides energy for cellular growth and metabolism. Potassium sulfate and magnesium chloride supply cations to activate pyocyanin production and to enhance pigment formation. Cethyl trimethyl ammonium bromide (cetrimide), the quaternary ammonium base present in the agar, inhibits the growth of most microorganisms with the exception of *Pseudomonas* spp. Pyocyanin production is typical of *P. aeruginosa* and is observed as a blue-green pigment that imparts a greenish colour into the medium.

Instruction for use

For laboratory use only.

Prepare the product to be examined according to the suitable Standard or Norm.

For the detection of *Pseudomonas aeruginosa* in non-sterile pharmaceutical products, European Pharmacopoeia recommends to prepare a 1:10 dilution with not less than 1 g of the product to be examined in Buffered sodium chloride peptone broth (Art. N° 84605.0500) and use 10 ml or a quantity corresponding to 1 g or 1 ml to inoculate a suitable amount of Tryptic soy broth (Art. N° 84675.0500); mix and incubate at 30-35°C for 18-24 hours.

Subculture on plates of *Pseudomonas selective (cetrimide) agar* and incubate at 30-35°C for 18-72 hours.

Growth of colonies indicates the possible presence of *P. aeruginosa*. This is confirmed by identification tests. A presumptive identification of the colonies should be done by observation of colony colour and fluorescence: green to blue-green colonies that fluoresce under UV light are considered presumptively positive for *P. aeruginosa*.

Limitations

- It is recommended that biochemical and/or serological tests be performed on colonies from pure culture for complete identification.

- Occasionally some enteric bacteria (e.g. *Klebsiella*, *Enterobacter*, *Citrobacter*, *Proteus*, *Providencia*), *Alkaligenes* and *Aeromonas* may exhibit growth with a slight yellowing of the medium; however, this colouration is easily distinguished from slight fluorescein production since this yellowing does not fluoresce.
- P.aeruginosa* may lose its fluorescence under UV lamp if the cultures are left at room temperature for a short time; however, fluorescence reappears when plates are re-incubated

Quality Control

Physical characteristics:

Appearance of powder	Yellowish, fine, homogeneous, hygroscopic powder
Appearance of prepared medium	Pale yellow, opalescent
pH (25°C)	7.2 ± 0.2

Microbiological characteristics:

Test strains	Incubation T° / t / At.	Inoculation method	Growth characteristics	Productivity Ratio
Growth promoting test according to Ph.Eur.				
<i>P.aeruginosa</i> ATCC 9027	30-35 °C / 18 h / AE	QT ≤ 100 CFU	Good growth with typical green-yellow fluorescent colonies	PR ≥ 0.7
Test for inhibitory properties, according to Ph.Eur.				
<i>E. coli</i> ATCC 8739	30-35 °C / 72 h / AE	EC ≥ 100 CFU	No growth	
Other quality control tests				
<i>P.aeruginosa</i> ATCC 14207	37 °C / 18 h / AE	EC	Good growth with typical green-yellow fluorescent colonies	
<i>P.aeruginosa</i> ATCC 10299	37 °C / 18 h / AE	EC	Good growth with typical green-yellow fluorescent colonies	
<i>A. calcoaceticus</i> ATCC 19606	37 °C / 72 h / AE	MM	No growth	
<i>S. aureus</i> ATCC 25923	37 °C / 72 h / AE	MM	No growth	

Notes

PR (Productivity Ratio): CFU obtained on the culture medium under test / CFU obtained on the Reference Batch

Incubation atmosphere AE: aerobic incubation

Inoculation method QT: quantitative surface plating method; EC: semi-quantitative, ecometric technique; MM: modified Miles-Misra surface drop method

Microbiological characteristics tested in accordance to Ph.Eur.

ATCC is a registered trade mark of American Type Culture Collection

References

- European Pharmacopoeia: 2.6.13 Microbiological examination of non-sterile products: tests for specified micro-organisms
- King, B.S., Ward, M.K.W. & Roney, D.E. (1954) - J. Lab. Clin. Med. **44**, 301.

Storage conditions

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Ordering information

Dehydrated medium:

84638.0500 Pseudomonas selective (Cetrimide) agar (Ph.Eur., USP, JP) Bottle of 500 g

Supplement:

84730.0001 Glycerol Bottle of 100 ml