

Pseudomonas agar base (ISO)

Code 84650.0500

Also known as

CN Pseudomonas agar, CN agar.

Intended use

Base medium for the isolation and enumeration of *Pseudomonas aeruginosa* in water samples and *Pseudomonas* spp. in meat, milk and milk products.

Formula * - Composition in g/L

Gelatin peptone.....	16.0
Casein hydrolysate.....	10.0
Potassium sulfate (anhydrous).....	10.0
Magnesium chloride (anhydrous).....	1.4
Agar	11.0

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

Final pH 7.1 ± 0.2 at 25 °C.

Instructions for preparation

Suspend 24.5 g in 500 ml of purified water and add 5 g of Glycerol (Art.: 84730.0001) for the preparation of CN Medium; omit the glycerol for the preparation of CFC and PP media. Bring to the boil with frequent shaking. Sterilise in the autoclave at 121 °C for 15 minutes and cool to 45-50°C.

CN medium (ISO 16266): dissolve the contents of one vial of CN selective supplement (Art.: 84745.0001) with 2 ml of sterile purified water/ethanol (1:1) and add to the cooled medium. Mix well and pour into sterile Petri dishes. Final pH 7.1 ± 0.2 at 25 °C.

CFC medium (ISO 13720): dissolve the contents of one vial of CFC selective supplement (Art.: 84746.0001) with 2 ml of sterile purified water/ethanol (1:1) and add to the cooled medium. Mix well and pour into sterile Petri dishes. Final pH 7.2 ± 0.2 at 25 °C.

PP medium (ISO/TS 11059): dissolve the contents of one vial of PP selective supplement (Art.: 84747.0001) with 5 ml of sterile purified water and add to the cooled medium. Mix well and pour into sterile Petridishes. Final pH 7.2 ± 0.2 at 25 °C.

Principle of the method and general information

Pseudomonas agar base supplemented with glycerol and CN selective supplement corresponds to the medium recommended by ISO 16266 for the isolation and enumeration of *Pseudomonas aeruginosa* in water samples; the medium base supplemented with CFC selective supplement corresponds to the medium recommended by ISO 13720 for the isolation and enumeration of *Pseudomonas* spp. in meat products; the medium base supplemented with PP selective supplement corresponds to the medium recommended by ISO/TS 11059 for the isolation and enumeration of *Pseudomonas* spp. in milk and milk products.

Gelatin peptone provides nitrogen, carbon 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. Pyocyanin production is typical of *P. aeruginosa* and is observed as a blue-green pigment that imparts a greenish colour into the medium.

The antimicrobials are added to the medium base as free-dried supplements (CN: cetrimide + nalidixic acid, CFC: cetrimide + fusidic acid + cephalorospirin, PP: pimaricin + penicillin G). Cetrimide (cethyl trimethyl ammonium bromide), a quaternary ammonium base, inhibits the growth of most microorganisms with the exception of *Pseudomonas* spp; the antibiotics fusidic acid, cephalosporin, penicillin G enhance the selectivity properties of plating media by inhibiting Gram positive and some Gram negative bacteria; pimaricin (natamycin) is an antifungal agent.

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Instructions for use

For laboratory use only.

Water (ISO 16266)

Filter through a 0.45 µm membrane the appropriate volume of water according to the degree of pollution expected. Transfer the filter membranes on the surface on plates prepared with CN selective supplement. Incubate at 36 °C ± 2° for 24-48 hours. Count all colonies that produce green/blue (pyocyanin) colour as confirmed *P.aeruginosa*. Consider presumptively as *P.aeruginosa* all the colonies fluorescent under a Wood's lamp and with typical smell and also the reddish brown colonies that do not fluoresce. Confirm the presumptive colonies using the medium containing acetamide, oxidase test and King's B medium.

Meat products (ISO 13720)

Transfer on the surface of one plate prepared with CFC selective supplement 0.1 ml of the initial suspension of the sample. Repeat these operation with subsequent dilutions taking two other CFC agar plates, using a new sterile pipette for each decimal dilution (if only one dilution is performed two plates shall be used).

Spread the liquid over the surface of the agar with a sterile spreader until the surface is completely dry.

Incubate the dishes at 25 °C for 40-48 h. Count the colonies on the plates containing less than 150 colonies and select random five colonies from each retained plate for confirmation tests.

Confirm the presence of *Pseudomonas* with oxidase test and fermentation of glucose. Colonies showing a positive oxidase reaction and absence of glucose fermentation shall be considered as *Pseudomonas* colonies.

Milk and milk products (ISO/TS 11059)

Transfer on the surface of the plate prepared with PP selective supplement 0.1 ml of the initial suspension. Repeat these operation with subsequent dilutions taking other PPA agar plate, using a new sterile pipette for each decimal dilution (if only one dilution is performed two plates shall be used).

Spread the liquid over the surface of the agar with a sterile spreader until the surface is completely dry.

Incubate the dishes at 25 °C for 48 ± 2 h. Count the colonies on the plates containing less than 150 colonies and select random five colonies from each retained plate for confirmation tests.

Confirm the presence of *Pseudomonas* with oxidase test and fermentation of glucose. Colonies showing a positive oxidase reaction and absence of glucose fermentation shall be considered as *Pseudomonas* colonies.

Limitations

- It is recommended that biochemical and/or serological tests be performed on pure culture for complete identification of the cultivated microorganisms.
- Some strains of *Pseudomonas aeruginosa* may fail to produce pyocyanin.

Quality Control

Physical characteristics:

Appearance of powder	White, fine, homogeneous, hygroscopic powder
Appearance of prepared medium	Pale yellow, limpid
pH (25°C)	7.1 ± 0.2

Microbiological characteristics:

Test strains	Incubation T° / t / At.	Inoculation method	Growth characteristics	Productivity rate
<i>P.aeruginosa</i> ATCC 27853	36 °C / 44 h / AE	QT	Good growth, yellow-green fluorescent colonies	PR ≥ 0.7
<i>P.aeruginosa</i> ATCC 14207	36 °C / 44 h / AE	EC	Good growth, yellow-green fluorescent colonies	
<i>E. coli</i> ATCC 25922	36 °C / 44 h / AE	MM	Totally inhibited	
<i>E. faecalis</i> ATCC 19433	36 °C / 44 h / AE	MM	Totally inhibited	

Notes

Medium supplementation: CN selective supplement (REF 84745.0001)

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

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Microbiological characteristics tested in accordance to ISO/TS 11133-2
ATCC is a registered trade mark of American Type Culture Collection

References

- ISO 16266:2006 Water quality – Detection and enumeration of *Pseudomonas aeruginosa* by membrane filtration.
- ISO 13720:2010 Meat and meat products — Enumeration of presumptive *Pseudomonas* spp.
- ISO/TS 11059:2009 Milk and milk products — Method for the enumeration of *Pseudomonas* spp.

Storage conditions

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+10 °C to 30 °C and <60% RH).

Ordering information

Dehydrated medium:

84650.0500 *Pseudomonas* agar base (ISO) Bottle of 500 g

Supplements:

84730.0001	Glycerol	Bottle of 100 ml
84745.0001	CN selective supplement	10 vials, each for 500 ml of complete medium
84746.0001	CFC selective supplement	10 vials, each for 500 ml of complete medium
84747.0001	PP selective supplement	10 vials, each for 500 ml of complete medium