

Technical Data Sheet

GranuCult™

Pseudomonas CFC/CN Agar (Base)

acc. ISO 13720 and ISO 16266

Ordering number: 1.07620.0500

For the detection and enumeration of *Pseudomonas spp.*

When supplemented with Pseudomonas CN Selective Supplement (article number 1.07624.0010) and glycerol, the medium complies with the specifications given by EN ISO 16266 for the detection and enumeration of *Pseudomonas aeruginosa* in water using the membrane filtration technique.

When supplemented with Pseudomonas CFC Selective Supplement (article number 1.07627.0010) the medium complies with the specifications given by EN ISO 13720 for the detection and enumeration of presumptive *Pseudomonas spp.* in meat and meat products including poultry.

Mode of Action

The peptone mixture in Pseudomonas Selective Agar Base allows growth of a broad spectrum of Pseudomonades. The amount of potassium sulfate and magnesium chloride supports enhances the pigment production of *Pseudomonas aeruginosa*.

By choosing the appropriate selective supplement and incubation temperature the medium becomes selective for *Pseudomonas spp.* (**CFC Agar**), or *Pseudomonas aeruginosa* (**CN Agar**).

Typical Composition

CN agar specified by EN ISO 16266		GranuCult™ Pseudomonas CFC/CN agar (base) with Pseudomonas CN supplement	
Enzymatic digest of gelatine	16 g/l	Enzymatic digest of gelatine	16 g/l
Enzymatic digest of casein	10 g/l	Casein hydrolysate	10 g/l
Potassium sulfate	10 g/l	Potassium sulfate	10 g/l
Magnesium chloride	1.4 g/l	Magnesium chloride anhydrous	1.4 g/l
Agar	11-18 g/l	Agar-agar*	11 g/l
Water	1000 ml/l	Water	n/a
Glycerol	10 ml	Glycerol (<i>to be added separately, not included in GranuCult™ formulation</i>)	10 ml
Supplements (added after autoclaving):			
Cetrimide	0.2 g/l	Cetrimide	0.2 g/l
Nalidixic acid	0.015 g/l	Nalidixic acid	0.015 g/l
pH at 25 °C	7.1 ± 0.2	pH at 25 °C	7.1 ± 0.2

* Agar-agar is equivalent to other different terms of agar.

CFC-Agar specified by EN ISO 13720		GranuCult™ Pseudomonas CFC/CN agar (base) with Pseudomonas CFC supplements	
Enzymatic digest of gelatin	16 g/l	Enzymatic digest of gelatin	16 g/l
Enzymatic digest of casein	10 g/l	Casein hydrolysate	10 g/l
Potassium sulfate	10 g/l	Potassium sulfate	10 g/l
Magnesium chloride	1.4 g/l	Magnesium chloride anhydrous	1.4 g/l
Agar	12-18 g/l	Agar-agar*	11 g/l
Water	1000 ml/l	Water	n/a
Supplements (added after autoclaving):			
Cephalothin	0.05 g/l	Cephalothin	0.05 g/l
Sodium fusidate	0.01 g/l	Sodium fusidate (Fucidin®)	0.01 g/l
Cetrimide	0.01 g/l	Cetrimide	0.01 g/l
pH at 25 °C	7.2 ± 0.2	pH at 25 °C	7.1 ± 0.2

* Agar-agar is equivalent to other different terms of agar.

Preparation

Dissolve 24.2 g in 500 ml of purified water, for Pseudomonas CN agar add 5 ml Glycerol (article number 104094), and heat to boiling until dissolved completely. Autoclave 15 minutes at 121 °C.

Cool the medium to 45-50 °C, and aseptically add the contents of one vial of Pseudomonas CFC Selective Supplement (article number 107627) or Pseudomonas CN Selective Supplement (article number 107624). Mix thoroughly and pour plates.

Do not keep the liquid medium (45-50 °C) longer than 4 hours. Do not re-melt the medium several times.

The prepared plates are clear and yellowish.

Experimental Procedure and Evaluation

Depend on the purpose for which the medium is used.

Following the procedure for direct enumeration given by EN ISO 16266, inoculate *Pseudomonas* CN agar plates using the membrane filtration technique.

Incubate at 34-38 °C for 40-48 hours aerobically.

Examine the membrane filters for growth after 20-24h and 40-48 h.

Count all colonies that produce a blue/green (pyocyanin) colour as confirmed *Pseudomonas aeruginosa*.

For counting and confirmation of fluorescent colonies (under UV light) and/or reddish brown pigmented colonies follow the procedure given by EN ISO 16266.

Following the procedure for direct enumeration given by EN ISO 13720, inoculate *Pseudomonas* CFC agar plates by means of a pipette 0.1 ml of the initial suspension or decimal dilution on the plate. Spread the liquid over the surface of the agar with a sterile spreader until the surface is completely dry.

Incubate at 24-26 °C for 40-48 hours aerobically.

Count the colonies on each plate and follow for confirmation the procedure e.g. given by EN ISO 13720.

Storage

Store at +15 °C to +25 °C, dry and tightly closed. Do not use clumped or discolored medium. Protect from UV light (including sun light). For *in vitro* use only.

According to EN ISO 13720 and EN ISO 16266, self-prepared *Pseudomonas* CN as well as CFC agar plates can be stored at +2 °C to +8 °C in the dark and protected against evaporation for up to 1 month.

Quality Control

Function	Control strains	Incubation	Reference medium	Method of control	Expected results
Performance test as <i>Pseudomonas</i> CFC agar in accordance with the current version of EN ISO 11133					
Productivity	<i>Pseudomonas fluorescens</i> ATCC® 13525	40-48 h at 24-26 °C	Tryptic Soy Agar (TSA)	Quantitative	Recovery ≥ 50 %, greenish colonies
	<i>Pseudomonas fragi</i> ATCC® 4973				Recovery ≥ 50 %, Milky colonies
Selectivity	<i>Escherichia coli</i> ATCC® 8739	40-48 h at 24-26 °C	-	Qualitative	Total inhibition
	<i>Escherichia coli</i> ATCC® 25922				

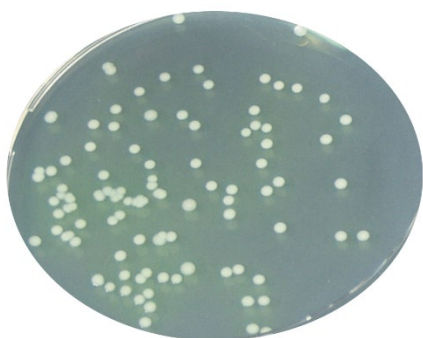
Please refer to the actual batch related Certificate of Analysis.

A recovery rate of 50 % is equivalent to a productivity value of 0.5.

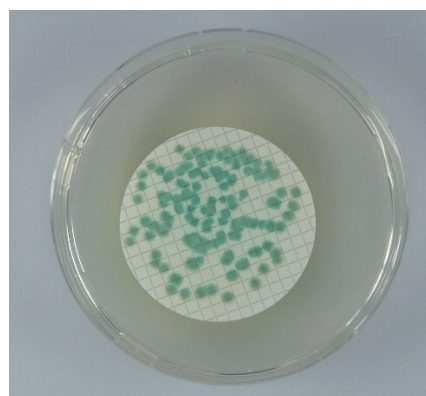
Function	Control strains	Incubation	Reference medium	Method of control	Expected results
Performance test as <i>Pseudomonas</i> CN agar in accordance with the current version of EN ISO 11133					
Productivity	<i>Pseudomonas aeruginosa</i> ATCC® 10145	40-48 h at 34-38 °C	Tryptic Soy Agar (TSA)	Quantitative with membrane filtration	Recovery ≥ 50 %, blue-green colonies with fluorescence under UV light
	<i>Pseudomonas aeruginosa</i> ATCC® 27853				
	<i>Pseudomonas aeruginosa</i> ATCC® 9027				
Selectivity	<i>Escherichia coli</i> ATCC® 8739	40-48 h at 34-38 °C	-	Qualitative	Total inhibition
	<i>Escherichia coli</i> ATCC® 25922				
	<i>Enterococcus faecalis</i> ATCC® 19433				

Please refer to the actual batch related Certificate of Analysis.

A recovery rate of 50 % is equivalent to a productivity value of 0.5.



Pseudomonas aeruginosa ATCC® 27853
on CFC agar



Pseudomonas aeruginosa ATCC® 27853
on CN agar

Literature

Druggan, P. and Iverson, C. (2012): Culture media for food spoilage bacteria of the order Pseudomonadales: *Pseudomonas*, *Acinetobacter* and *Psychrobacter* spp. In: Handbook of Culture Media for Food and Water Microbiology. (Corry, J.E.L., Curtis, G.D.W. and Baird, R.M. eds)., pp. 482-502. Royal Society of Chemistry, Cambridge, UK.

Goto, S., and Enomoto, S. (1970): Nalidixic Acid Cetrinide Agar. A New Selective Plating Medium for the Selective Isolation of *Pseudomonas aeruginosa*. Japan. J. Microbiol. **14**: 65 - 72.

ISO International Standardisation Organisation. Meat and meat products - Enumeration of presumptive *Pseudomonas* spp. EN ISO 13720:2010.

ISO International Standardisation Organisation. Water quality -- Detection and enumeration of *Pseudomonas aeruginosa* -- Method by membrane filtration. EN ISO 16266:2006.

ISO International Standardisation Organisation. Microbiology of food, animal feed and water - Preparation, production, storage and performance testing of culture media. EN ISO 11133:2014.

Mead, G.C. and Adams, B.W. (1977): A selective medium for the rapid isolation of *Pseudomonas* associated with poultry meat spoilage. Br. Poult. Sci. **18**: 661 - 670.

Watkins, J. and Sartory, D. (2012): Culture media for the isolation and enumeration of microorganisms from water. In: Handbook of Culture Media for Food and Water Microbiology. (Corry, J.E.L., Curtis, G.D.W. and Baird, R.M. eds)., pp. 605-618. Royal Society of Chemistry, Cambridge, UK.

Ordering Information

Product	Cat. No.	Pack size
GranuCult™ <i>Pseudomonas</i> CFC/CN Agar (Base) acc. ISO 13720 and ISO 16266	1.07620.0500	500 g
<i>Pseudomonas</i> CFC Selective Supplement (Cetrimide, Fucidin® [= Sodium Fusidate], Cephalotin)	1.07627.0010	10 vials
<i>Pseudomonas</i> CN Selective Supplement (Cetrimide, Nalidixic acid)	1.07624.0010	10 vials
Glycerol 85%	1.04094.0500	500 ml
EZ-PAK Filters 0,45µm 47 mm white, gridded	EZHAWG474	4 bands of 150 filters
S-PAK mixed cellulose ester filter 0.45µm 47 mm white, gridded	HAWG047S6	600 individually sealed filters, sterile
King agar B (Base)	1.10991.0500	500 g
Bactident® Oxidase - For the detection of cytochrome oxidase in microorganisms	1.13300.0001	50 strips

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