Pseudomonas Agar Base
SKU: 700004474, 700004475, 700004476, 700004477
(NCM0083)

Intended Use
Pseudomonas Agar Base is used for the selective and differential isolation of *Pseudomonas* sp. using CN or CFC supplementation and is not intended for use in the diagnosis of disease or other conditions in humans.

Product Summary and Explanation
Pseudomonas Agar Base was developed to be supplemented with CN NCM4008 (Cetrimide & Nalidixic Acid) as per ISO 16266:2006, or with CFC NCM4023 (Cetrimide, Fusidic Acid, & Cephalothin) as per ISO 13720:2010. This base formula is a modification of King's A Medium.

The CN Supplement is recommended for the isolation of *Pseudomonas aeruginosa,* where the addition of Nalidixic Acid and the reduction of Cetrimide improved recovery. Pseudomonas Agar Base, with the addition of CN Supplement, demonstrated enhanced pigment formation of *Ps. aeruginosa* and increased inhibition of *Klebsiella*, *Proteus*, and *Providencia* spp. Pseudomonas Agar Base with the addition of CFC Supplement is recommended for the selective isolation of *Pseudomonas* spp. Mead and Adams reduced Cetrimide, permitting growth of all pigmented and non-pigmented psychrophilic pseudomonads. The antimicrobics, Fusidic Acid and Cephalothin, were added to increase the selectivity of the medium. This formulation is used for *Pseudomonas* spp. from chilled foods and processing plants.

Pseudomonas Agar Base is recommended by ISO for the enumeration of *Pseudomonas* spp. from meat and meat products according to ISO 13720, and for the detection of enumeration of *Pseudomonas aeruginosa* in water according to ISO 16266.

Typical Formulation

Enzymatic Digest of Gelatin	16.0 g/L
Enzymatic Digest of Casein	10.0 g/L
Potassium Sulfate	10.0 g/L
Magnesium Chloride	1.4 g/L
Agar	11.0 g/L

Final pH: 7.1 ± 0.2 at 25°C
Formula is adjusted and/or supplemented as required to meet performance specifications.

Supplements
10 mL Glycerol

NCM4008	CN Supplement
NCM4023	CFC Supplement

Precaution
Refer to SDS

Preparation
CFC Formula
1. Suspend 48.4 grams of the medium and 10 mL of glycerol in one liter of purified water and bring to a boil.
2. Autoclave at 121°C for 15 minutes.
3. Cool to 45 – 50°C.
4. Aseptically add 2 vials of NCM4023-0.5* or 700004886 CFC Supplement, each reconstituted using 5 mL of 50% ethanol.

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*Larger vials may be available. Please see appropriate supplement data sheet for availability and preparation instructions

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2. Autoclave at 121°C for 15 minutes.
3. Cool to 45 – 50°C.
4. Aseptically add 2 vials of NCM4008-0.5* or 700003738 CN Supplement, each reconstituted using 5 mL of sterile deionized/RO water.

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Test Procedure

Food, and Environmental Samples

1. For the isolation of Pseudomonads in food, and environmental samples prepare formula with CFC Supplement (NCM4023).
2. Prepare food samples by diluting 1 in 5, or 1 in 10 with 1% sterile Peptone Water (NCM0096 or 400000802) and place in a stomacher or laboratory blender.
3. Pipette 0.5 mL or 1 mL of the homogenate onto the prepared medium using the spread plate technique. Inoculate water and swab samples directed onto the surface of the medium.
4. Incubate at 25°C and examine for growth and fluorescence at 24 and 48 hours, using both white and UV light.

Water Samples

1. For the isolation of Pseudomonads in water samples prepare formula with CN Supplement (NCM4008).
2. Inoculate Pseudomonas CN agar plates using the membrane filtration technique.
3. Incubate at 34-38 °C for 40-48 hours aerobically.
4. Examine the membrane filters for growth after 20-24h and 40-48 h.
5. Count all colonies that produce a blue/green (pyocyanin) color as confirmed Pseudomonas aeruginosa.
6. For counting and confirmation of fluorescent colonies (under UV light) and/or reddish-brown pigmented colonies follow the procedure given by EN ISO 16266

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is clear, may have slight precipitate and light beige.

Expected Cultural Response: The cultures were incubated aerobically at 34-38°C CN supplement; 24-26°C CFC supplement and examined for growth at 40-48 hours and fluorescence under white, and long-wave UV light.

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CFC Supplement

MICROORGANISM	APPROX. INOCULUM	EXPECTED RESULTS		
		Growth	Color	UV
Escherichia coli ATCC® 25922	>10^4	Complete inhibition	-	-
Escherichia coli ATCC® 8739	>10^4	Complete inhibition	-	-
Pseudomonas fluorescens ATCC® 13525	50-200	>50%	Yellowish green to green	+
Pseudomonas fragii ATCC® 4973	50-200	>50%	Yellowish green to green	-
Pseudomonas putida ATCC® 17391	50-200	>50%	Straw	+
Staphylococcus aureus ATCC® 25923	>10^4	Complete inhibition	-	-

CN Supplement

MICROORGANISM	APPROX. INOCULUM	EXPECTED RESULTS		
		Growth	Color	UV
Escherichia coli ATCC® 25922	>10^4	Complete inhibition	-	-
Escherichia coli ATCC® 8739	>10^4	Complete inhibition	-	-
Enterococcus faecalis ATCC® 29212	>10^4	Complete inhibition	-	-
Enterococcus faecalis ATCC® 19433	>10^4	Complete inhibition	-	-
Pseudomonas aeruginosa ATCC® 27853	50-200	>50%	Yellowish green to green	+
Pseudomonas aeruginosa ATCC® 9027	50-200	>50%	Yellowish green to green	+
Pseudomonas aeruginosa ATCC® 10145	50-200	>50%	Yellowish green to green	+
Pseudomonas putida ATCC® 17391	50-200	Complete inhibition	-	-

The organisms listed are the minimum that should be used for quality control testing.

Results

The presence of green colonies and fluorescence is presumptive evidence of *Pseudomonas aeruginosa*. Other *Pseudomonas* spp. colonies may have a straw color with and without fluorescence. Further tests are necessary for confirmation of *Pseudomonas aeruginosa* and *Pseudomonas* spp.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container.

Limitations of the Procedure

1. Some strains of *Pseudomonas aeruginosa* may fail to produce pyocyanin.
2. It is not expected, but Enterobacteriaceae may also grow on this medium.

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Storage

Store dehydrated culture media at 2 – 30°C away from direct sunlight. Once opened and recapped, place the container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

References

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