Pseudomonas Agar Base (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 X107 (Cetrimide & Nalidixic Acid) as per ISO 16266-2:2018, or with CFC X108 (Cetrimide, Fusidate, & Cephaloridine) 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, Fusidate and Cephaloridine, 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**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzymatic Digest of Gelatin</td>
<td>16.0 g/L</td>
</tr>
<tr>
<td>Enzymatic Digest of Casein</td>
<td>10.0 g/L</td>
</tr>
<tr>
<td>Potassium Sulfate</td>
<td>10.0 g/L</td>
</tr>
<tr>
<td>Magnesium Chloride</td>
<td>1.4 g/L</td>
</tr>
<tr>
<td>Agar</td>
<td>11.0 g/L</td>
</tr>
<tr>
<td>Final pH: 7.1 ± 0.2 at 25°C</td>
<td></td>
</tr>
</tbody>
</table>

Formula may be adjusted and/or supplemented as required to meet performance specifications.

**Supplement/Liter**

10 mL Glycerol (when supplementing with CN under ISO 16266)

**CN Supplement (X107)** – 1 vial per 500mL

- 200 mg/L Cetrimide
- 15 mg/L Nalidixic Acid

**CFC Supplement (X108)** – 1 vial per 500mL

- 10 mg/L Cetrimide
- 10 mg/L Fusidate
- 50 mg/L Cephaloridine

**Precaution**
Refer to SDS

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Technical Specification Sheet

NEOGEN
Culture Media

620 Lesher Place • Lansing, MI 48912
800-234-5333 (USA/Canada) • 517-372-9200
foodafety@neogen.com • foodsafety.neogen.com
1. Suspend 48.4 g 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 CFC Supplement (X108) and mix well before dispensing.

**CN Formula**
1. Suspend 48.4 g of the medium 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 CN Supplement (X107) and mix well before dispensing.

**Test Procedure**

**Food, and Environmental Samples**
1. For the isolation of Pseudomonads in food, and environmental samples prepare formula with CFC Supplement (X108).
2. Prepare food samples by diluting 1 in 5, or 1 in 10 with 1% sterile Peptone Water (# 7365) 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 (X107).
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.
## CFC Supplement

<table>
<thead>
<tr>
<th>MICROORGANISM</th>
<th>APPROX. INOCULUM</th>
<th>EXPECTED RESULTS</th>
<th>GROWTH</th>
<th>COLOR</th>
<th>UV</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em> ATCC® 25922</td>
<td>&gt;10⁴</td>
<td>Complete inhibition</td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Escherichia coli</em> ATCC® 8739</td>
<td>&gt;10⁴</td>
<td>Complete inhibition</td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Pseudomonas fluorescens</em> ATCC® 13525</td>
<td>50-200</td>
<td>&gt;50%</td>
<td>Yellowish green to green</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas fragii</em> ATCC® 4973</td>
<td>50-200</td>
<td>&gt;50%</td>
<td>Yellowish green to green</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas putida</em> ATCC® 17391</td>
<td>50-200</td>
<td>&gt;50%</td>
<td>Straw</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> ATCC® 25923</td>
<td>&gt;10⁴</td>
<td>Complete inhibition</td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
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## CN Supplement

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<tr>
<td><em>Escherichia coli</em> ATCC® 8739</td>
<td>&gt;10⁴</td>
<td>Complete inhibition</td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em> ATCC® 29212</td>
<td>&gt;10⁴</td>
<td>Complete inhibition</td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em> ATCC® 19433</td>
<td>&gt;10⁴</td>
<td>Complete inhibition</td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em> ATCC® 27853</td>
<td>50-200</td>
<td>&gt;50%</td>
<td>Yellowish green to green</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em> ATCC® 9027</td>
<td>50-200</td>
<td>&gt;50%</td>
<td>Yellowish green to green</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em> ATCC® 10145</td>
<td>50-200</td>
<td>&gt;50%</td>
<td>Yellowish green to green</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas putida</em> ATCC® 17391</td>
<td>50-200</td>
<td>Complete inhibition</td>
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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.

**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**