CLOSTRIDIUM PERFRINGENS AGAR BASE (9188)

Intended Use
Clostridium Perfringens Agar Base is used with selective and differential supplements for the recovery of Clostridium perfringens in a laboratory setting. Clostridium Perfringens Agar Base is not intended for use in the diagnosis of disease or other conditions in humans.

Product Summary and Explanation
Clostridium Perfringens Agar Base is a nutritious base medium used in the preparation of SFP Agar and TSC Agar. Depending upon the formula, supplements are added to increase the selectivity of the medium. Shahidi-Ferguson Perfringens (SFP) Agar is based on the formula by Shahidi and Ferguson and incorporates the supplements, Polymyxin B Sulfate and Kanamycin. Tryptose Sulphite Cycloserine (TSC) Agar is developed with the same base as SFP Agar, with the addition of Cycloserine. If desired, Egg Yolk Emulsion can be added to either formula.

Principles of the Procedure
Enzymatic Digest of Casein, Enzymatic Digest of Soybean Meal, and Yeast Extract provide nitrogen, vitamins, and amino acids in Clostridium Perfringens Agar Base. Sodium Metabisulfite increases the aerotolerance of Clostridium perfringens by acting as oxygen scavengers. Ferric Ammonium Citrate is used to differentiate sulfite-reducing Clostridium perfringens, allowing visualization of hydrogen sulfide which produce black colonies on the medium. Agar is used to solidify the medium. The antimicrobic supplements provide a high degree of selectivity to the medium. The addition of Egg Yolk Emulsion may produce lecithinase activity, causing an opaque zone around the colony.

Formula / Liter
<table>
<thead>
<tr>
<th>Supplement, # 7982 (If desired)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg Yolk Emulsion, 100 mL</td>
</tr>
<tr>
<td>50% Solution</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Enzymatic Digest of Casein</th>
<th>15 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzymatic Digest of Soybean Meal</td>
<td>5 g</td>
</tr>
<tr>
<td>Yeast Extract</td>
<td>5 g</td>
</tr>
<tr>
<td>Sodium Metabisulfite</td>
<td>1 g</td>
</tr>
<tr>
<td>Ferric Ammonium Citrate</td>
<td>1 g</td>
</tr>
<tr>
<td>Agar</td>
<td>14 g</td>
</tr>
<tr>
<td>Final pH: 7.6 ± 0.2 at 25°C</td>
<td></td>
</tr>
</tbody>
</table>

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

Precautions
1. For Laboratory Use Only.
2. HARMFUL. May be harmful if swallowed or inhaled. Irritating to eyes, respiratory system, and skin.

Directions for SFP and TSC
1. Suspend 41 g of the medium in 900 mL of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes. Cool to 45 - 50°C.
4. Add 100 mL of Egg Yolk Emulsion, 50% (# 7982) and the desired antimicrobic supplement:
   - TSC Agar: Add 10 mL of a sterile 4% solution of Cycloserine.
   - SFP Agar: Add 10 mL of a sterile solution containing 3 mg of Polymyxin B Sulfate and 12 mg of Kanamycin.

Overlay Agar
1. Suspend 41 grams of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes. Cool to 45 - 50°C.
4. Overlay agar does not get supplemented with Egg Yolk Emulsion. Overlay agar is held at 45 -50°C until used, and should be poured right after inoculation.
Quality Control Specifications

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

Prepared Appearance: Prepared medium is light to medium beige and trace to slightly hazy.

Expected Cultural Response: Cultural response on Clostridium Perfringens Agar Base, prepared as TSC Agar and SFP Agar, supplemented as required. After inoculation of the base layer, medium was covered with overlay agar. Clostridium species were incubated under anaerobic conditions and all others under aerobic conditions at 35°C for 18 - 24 hours of incubation.

TSC Agar

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Approx. Inoculum (CFU)</th>
<th>Expected Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clostridium perfringens ATCC® 10543</td>
<td>10 - 300</td>
<td>Suppressed to inhibited, black colonies where present</td>
</tr>
<tr>
<td>Clostridium perfringens ATCC® 13124</td>
<td>10 - 300</td>
<td>Black colonies</td>
</tr>
<tr>
<td>Clostridium novyi ATCC® 17861</td>
<td>10 - 300</td>
<td>Suppressed to inhibited</td>
</tr>
<tr>
<td>Clostridium tetani ATCC® 19406</td>
<td>10 - 300</td>
<td>Suppressed to inhibited</td>
</tr>
<tr>
<td>Bacillus cereus ATCC® 11778</td>
<td>300 - 1000</td>
<td>Suppressed to inhibited</td>
</tr>
<tr>
<td>Escherichia coli ATCC® 25922</td>
<td>300 - 1000</td>
<td>Suppressed to inhibited</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa ATCC® 27853</td>
<td>300 - 1000</td>
<td>Suppressed to inhibited</td>
</tr>
</tbody>
</table>

SFP Agar

<table>
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<tr>
<th>Microorganism</th>
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<td>Pseudomonas aeruginosa ATCC® 27853</td>
<td>300 - 1000</td>
<td>Suppressed to inhibited</td>
</tr>
</tbody>
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Test Procedure

For a complete discussion on the isolation and identification of *C. perfringens* and other anaerobic bacteria refer to specific procedures in appropriate references.3,4

Results

*Clostridium perfringens* produce black colonies on TSC Agar and SFP Agar. If Egg Yolk Emulsion is added, colonies may have an opaque halo around the black colony due to lecithinase activity. All black colonies should be confirmed.3,4,5 Cultures which are not overlaid with agar are unlikely to produce black colonies.

Storage

Store sealed bottle containing dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

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 when stored as directed.
Limitations of the Procedure

1. Both black lecithinase-positive and black lecithinase-negative colonies should be considered as presumptive Clostridium perfringens on TSC or SFP Agars. Perform confirmatory tests.
2. Egg yolk positive facultative anaerobes may grow on SFP Agar producing completely opaque plates, and covering up the egg yolk reaction of Clostridium perfringens.

Packaging

<table>
<thead>
<tr>
<th>Clostridium Perfringens Agar Base</th>
<th>Code No.</th>
<th>9188A</th>
<th>500 g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>9188B</td>
<td>2 kg</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9188C</td>
<td>10 kg</td>
</tr>
<tr>
<td>Egg Yolk Emulsion</td>
<td></td>
<td>7982</td>
<td>100 mL</td>
</tr>
</tbody>
</table>

References

5. www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalmanualBAM/default.htm.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (517)372-9200 or fax us at (517)372-2006.