**Bifidobacterium Selective Count Agar Base (BSC Propionate Agar Base)**

**Intended Use:**
Recommended for enumeration of presumptive Bifidobacteria by colony count technique from milk products.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptone</td>
<td>10.000</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>1.000</td>
</tr>
<tr>
<td>Potassium dihydrogen phosphate</td>
<td>3.000</td>
</tr>
<tr>
<td>Dipotassium hydrogen phosphate</td>
<td>4.800</td>
</tr>
<tr>
<td>Ammonium sulphate</td>
<td>3.000</td>
</tr>
<tr>
<td>Magnesium sulphate heptahydrate</td>
<td>0.200</td>
</tr>
<tr>
<td>L-Cysteine HCl monohydrate</td>
<td>0.500</td>
</tr>
<tr>
<td>Sodium propionate</td>
<td>15.000</td>
</tr>
<tr>
<td>Galactooligosaccharide</td>
<td>10.000</td>
</tr>
<tr>
<td>Agar</td>
<td>15.000</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>6.3±0.2</td>
</tr>
</tbody>
</table>

**Directions**

Suspend 62.35 grams (the equivalent weight of dehydrated medium per litre) in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at (115±3°C) for 15 minutes. Cool to 45-50°C. For selective isolation of Bifidobacteria add contents of 2 vials of Bifido Selective Supplement A (FD250). Mix carefully to avoid the formation of air bubbles and pour into sterile Petri plates or dispense as desired.

**Note:** This medium being sensitive to heat, excessive heat treatment may therefore indicatively influence the properties of the medium. For more selectivity Glacial acetic acid (Bifido Selective Supplement B FD251) may also be added. After addition of Bifido Selective Supplement B (FD251) pH of the medium will shift to the acidic side, which does not affect the performance of the medium.

**Principle And Interpretation**

Bifidobacteria Selective Count Agar Base is specifically prepared for selective enumeration of Bifidobacteria in fermented milks and fermented milk drinks living together with lactic acid bacteria. Bifidobacteria Selective Count Agar Base contains highly purified Galactooligosaccharides, which is one of the most excellent Bifidobacteria growth substances. Cysteine hydrochloride helps in creating reduced conditions required for the growth of Bifidobacteria. Tryptone acts as rich nitrogen source. The antibiotic mupirocin inhibits the growth of most lactic acid bacteria commonly used in fermented and non-fermented dairy products. Freshly prepared culture media not exposed to direct sunlight is recommended (2).

**Test Procedure:** Before opening the sample container, clean the external surface surrounding of the area from which the test sample is to be taken, in order to remove any material that might contaminate the sample. Weigh 90 gm of diluent in each of the 250 ml pre-sterilized bottles. Close the bottles. Weigh 10 gm of the test sample directly into the bottle with the diluent at 45°C. To dissolve the test sample, swirl slowly to wet the powder. The time between ending the preparation of the primary dilution until addition of culture medium shall not exceed 15 min. Immediately after solidification of the medium, invert all Petri dishes in the anaerobic culture jar or anaerobic incubator at 37°C for 72 hrs ± 3 hrs. Count the colonies after incubation. Bifidobacterial colonies are recognized by their whitish colour and acetic acid odour. Some of the bifidobacterial strains may appear in different colony size as well as colony appearance on the same plate (2).

**Type of specimen**

Dairy samples

Please refer disclaimer Overleaf.
Specimen Collection and Handling
For dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,6).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions
Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations
1. Some Bifidobacterias are extremely fastidious that may show poor growth due to nutritional variations and selectivity.
2. Further biochemical and serological testing is required for complete identification.

Performance and Evaluation
Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control
Appearance
Cream to yellow homogeneous free flowing powder
Gelling
Firm, comparable with 1.5% Agar gel
Colour and Clarity of Prepared medium
Yellow coloured opalescent gel forms in Petri plates
Reaction
Reaction of 6.20% w/v aqueous solution at 25°C. pH : 6.3±0.2
pH
6.10-6.50
Cultural Response
Cultural characteristics observed with added Bifido Selective Supplement A under anaerobic conditions, after an incubation at 35-37°C for 48-72 hours.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
<th>Growth with FD250</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bifidobacterium breve ATCC 15100</td>
<td>50-100</td>
<td>luxuriant</td>
<td>Good-luxuriant</td>
</tr>
<tr>
<td>Lactococcus lactis ATCC 19435 (00016*)</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>inhibited</td>
</tr>
<tr>
<td>Lactococcus cremoris ATCC 50-100</td>
<td>19257</td>
<td>good-luxuriant</td>
<td>inhibited</td>
</tr>
<tr>
<td>Lactobacillus acidophilus ATCC 4356 (00098*)</td>
<td>50-100</td>
<td>good</td>
<td>inhibited</td>
</tr>
</tbody>
</table>

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life
Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Use before expiry date on the label. Product performance is best if used within stated expiry period.

Disposal
User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

Please refer disclaimer Overleaf.
Reference


