**Intended use**
Recommended for the differentiation of *Bifidobacterium* and *Lactobacillus* species from clinical and non-clinical samples.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone special</td>
<td>23.000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.000</td>
</tr>
<tr>
<td>M-Protein powder #</td>
<td>5.000</td>
</tr>
<tr>
<td>Chromogenic mixture</td>
<td>10.480</td>
</tr>
<tr>
<td>Agar</td>
<td>16.000</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>7.2±0.2</td>
</tr>
</tbody>
</table>

**Formula adjusted, standardized to suit performance parameters**

# - Equivalent to Milk powder

**Directions**
Suspend 59.48 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

**Principle And Interpretation**
The genus *Bifidobacterium* is the third most numerous bacterial populations found in the human intestine after *Bacteroides* and *Eubacterium*. It is an anaerobic bacteria that makes up the gut microbial flora. It resides in the colon and have health benefits for their hosts. Bifidobacteria are also associated with lower incidences of allergies (3,4). Bifidobacterium Agar is used for the cultivation and maintenance of *Bifidobacterium* species (2).

Peptone special provides nitrogenous and carbonaceous compounds, long chain amino acids, vitamins and other essential growth nutrients. Sodium chloride maintains osmotic balance. M-protein powder aids in detecting casein hydrolysis activity which is exhibited by *Bifidobacterium breve*. A halo zone is observed around the colony in case of casein hydrolysis. The indicator system in the chromogenic mixture helps in distinguishing between *Lactobacillus* and *Bifidobacterium* species. *Lactobacillus* species usually produce green colonies with opaque zone. *Bifidobacterium infantis* produces dark blue to bluish green colonies. Agar serves as a solidifying agent.

**Type of specimen**
Clinical samples - Faeces; Food and dairy samples.

**Specimen Collection and Handling**
For clinical samples follow appropriate techniques for handling specimens as per established guidelines (5,6).
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,7,8).
After use, contaminated materials must be sterilized by autoclaving before discarding.

**Warning and Precautions**
In Vitro diagnostic Use only. 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 clinical specimens. Safety guidelines may be referred in individual safety data sheets.

**Limitations**
1. Due to variable nutritional requirements, some strains may show poor growth on this medium.
2. Slight colour variation may be observed depending upon the utilization of the substrate by the organism.
3. *Bifidobacterium* species are strict anaerobes, hence condition must be appropriately maintained.
**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 1.6% Agar gel

**Colour and Clarity of prepared medium**
Reddish orange coloured clear to slightly opalescent gel forms in Petri plates

**Reaction**
Reaction of 5.95% w/v aqueous solution at 25°C. pH :7.2±0.2

**Cultural Response**
Cultural characteristics observed after an incubation at 35-37°C for 48 hours under anaerobic conditions.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
<th>Recovery</th>
<th>Colour of colony</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bifidobacterium infantis</em> ATCC 25962</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
<td>Dark blue-bluish green</td>
</tr>
<tr>
<td><em>Bifidobacterium breve</em> ATCC 15698</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
<td>Red-Pink with halo zone</td>
</tr>
<tr>
<td><em>Lactobacillus plantarum</em> ATCC 8014</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
<td>Green colonies w/ halo zone</td>
</tr>
<tr>
<td><em>Lactobacillus fermentum</em> ATCC 9338</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
<td>Pink without halo zone</td>
</tr>
</tbody>
</table>

**Storage and Shelf Life**
Store the dehydrated and the prepared medium in a tightly closed container 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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (5,6).

**Reference**
Disclaimer:

User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal or therapeutic use but for laboratory, diagnostic, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.