HYA Agar is used for differentiation of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* on the basis of colony morphology from yoghurt cultures.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef extract</td>
<td>1.000</td>
</tr>
<tr>
<td>Proteose peptone</td>
<td>10.000</td>
</tr>
<tr>
<td>Dextrose</td>
<td>2.500</td>
</tr>
<tr>
<td>Galactose</td>
<td>2.500</td>
</tr>
<tr>
<td>Lactose</td>
<td>5.000</td>
</tr>
<tr>
<td>Agar</td>
<td>15.000</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>6.8±0.2</td>
</tr>
</tbody>
</table>

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

**Directions**

Suspend 36 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 20 minutes. Mix well and pour into sterile Petri plates.

**Principle And Interpretation**

Yoghurt is a fermentable milk product in which *Streptococcus thermophilus* and *Lactobacillus bulgaricus* are the essential microbial species and are active in a symbiotic relationship. The large number of media proposed for lactic acid bacteria, particularly for Streptococci and lactobacilli is indicative of the difficulties encountered in growing some strains of these organisms. The choice of medium is governed to some extent by the particular strains under study and therefore by products or habitat. In general, lactic acid bacteria are tolerant to low pH, they can be very sensitive to other adverse conditions. Samples to be examined for numbers of viable lactic acid bacteria should not be frozen prior to analysis (2).

Porubcan and Sellars (1) described this medium on which *L.bulgaricus* grow as diffuse, low mass colonies (2-10 mm in diameter) and *S.thermophilus* as discrete high mass colonies (1-3 mm in diameter). To obtain optimum consistency, flavour and odour, many investigators claim that the two species should be present in about equal numbers in the culture. Dominance by either species can cause defects. Because of the emphasis on maintaining balance between coccus and rods, techniques are needed to determine the relative proportion of *S.thermophilus* and *L.bulgaricus* when grown together in milk culture.

Differentiation of two species on HYA Agar is based on colony morphology. Also this media is recommended by APHA (2).

Beef extract and proteose peptone provides necessary nitrogenous nutrients required for growth of two species. The sugars dextrose, galactose, lactose serve as energy sources.

**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, clear to slightly opalescent gel forms in Petri plates

- **Reaction**
  Reaction of 3.6% w/v aqueous solution at 25°C. pH : 6.8±0.2

- **pH**
  6.60-7.00

- **Cultural Response**
  M601: Cultural characteristics observed after an incubation at 35-37°C for 24-48 hours .
Organism | Cultural Response | Growth
--- | --- | ---
*Lactobacillus bulgaricus* | *ATCC 11842* | luxuriant
*Streptococcus thermophilus* | *ATCC 14485* | luxuriant

**Storage and Shelf Life**

Store below 30°C in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

**Reference**


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