B12 Culture Agar (E.coli Maintenance Medium) M185

B12 Culture Agar (E.coli Maintenance Medium) is recommended for propagation, cultivation and maintenance of *Escherichia coli* mutant 113.3 Davis ATCC 11105 used in microbiological assay of Vitamin B12.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein enzymic hydrolysate</td>
<td>5.000</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>5.000</td>
</tr>
<tr>
<td>Liver extract</td>
<td>0.050</td>
</tr>
<tr>
<td>Sucrose</td>
<td>12.000</td>
</tr>
<tr>
<td>Monopotassium phosphate</td>
<td>0.500</td>
</tr>
<tr>
<td>Magnesium sulphate</td>
<td>0.200</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>0.100</td>
</tr>
<tr>
<td>Ferrous sulphate</td>
<td>0.001</td>
</tr>
<tr>
<td>Agar</td>
<td>15.000</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>7.0±0.2</td>
</tr>
</tbody>
</table>

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

**Directions**

Suspend 37.85 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.

**Principle And Interpretation**

B12 Culture Agar (E.coli Mutant Culture Agar) is recommended for cultivation and maintenance of *E.coli* ATCC 11105 (1). Casein enzymic hydrolysate provides nitrogen and amino acids for the growth of *E. coli* 113.3. Yeast extract is vitamin source in the medium while sucrose is carbon and energy source. Potassium salt provides buffering capacity to the medium and sodium, magnesium and ferrous salts control permeability of bacterial cells. Liver extract enriches the medium for growth of *E. coli* as well as inhibits gram positive bacteria.

Stock cultures of *E.coli* ATCC 11105 are prepared by stab cultures are made at least three times in a week. Do not use the culture for preparing assay inoculum if it is over 4 days old. Before using a fresh culture for assay, make at least 10 successive transfers of the culture in 15 days period. Incubate the cultures for 16-24 hours at 35°C but hold constant within ±0.5°C. After incubation store at 2-8°C.

**Quality Control**

**Appearance**
Cream to yellow coloured homogeneous free flowing powder

**Gelling**
Firm, comparable with 1.5% Agar gel

**Colour and Clarity of prepared medium**
Yellow coloured slightly opalescent gel forms in tubes as slants.

**Reaction**
Reaction of 3.79% w/v aqueous solution at 25°C. pH : 7.0±0.2

**pH**
6.80-7.20

**Cultural Response**
Cultural characteristics observed after an incubation at 35 - 37°C for 18 - 48 hours.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
<th>Recovery</th>
</tr>
</thead>
</table>

Please refer disclaimer Overleaf.
Cultural Response

*Escherichia coli ATCC 11105*

50-100 luxuriant >=70%

Storage and Shelf Life

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

Reference


Disclaimer:

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