Middlebrook 7H9 Agar Base

Middlebrook 7H9 Agar Base is recommended for isolation, cultivation and sensitivity testing of *Mycobacterium tuberculosis*.

**Composition**

**Ingredients**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonium sulphate</td>
<td>0.500</td>
</tr>
<tr>
<td>Sodium glutamate</td>
<td>0.500</td>
</tr>
<tr>
<td>Sodium citrate</td>
<td>0.100</td>
</tr>
<tr>
<td>Pyridoxine</td>
<td>0.001</td>
</tr>
<tr>
<td>Biotin</td>
<td>0.0005</td>
</tr>
<tr>
<td>Disodium phosphate</td>
<td>2.500</td>
</tr>
<tr>
<td>Monopotassium phosphate</td>
<td>1.000</td>
</tr>
<tr>
<td>Ferric ammonium citrate</td>
<td>0.040</td>
</tr>
<tr>
<td>Magnesium sulphate</td>
<td>0.050</td>
</tr>
<tr>
<td>Calcium chloride</td>
<td>0.0005</td>
</tr>
<tr>
<td>Zinc sulphate</td>
<td>0.001</td>
</tr>
<tr>
<td>Copper sulphate</td>
<td>0.001</td>
</tr>
<tr>
<td>Malachite green</td>
<td>0.001</td>
</tr>
<tr>
<td>Agar</td>
<td>15.000</td>
</tr>
</tbody>
</table>

**Final pH (at 25°C)**

6.6±0.2

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

**Directions**

Suspend 9.85 grams in 450 ml distilled water. 1 ml glycerol may be added if desired. 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 and aseptically add 1 vial of Middlebrook OADC Growth Supplement (FD018). Mix well and distribute as desired.

**Principle And Interpretation**

Solid media for Mycobacterial cultivation may be egg-based (Lowenstein Jensen Media) or agar-based (Middlebrook Media) (1). Dubos and Middlebrook (2) developed various formulations containing oleic acid and albumin, which protect Mycobacterium from toxic agents, helping for the growth of tubercle bacilli. Middlebrook 7H9 Agar Base developed by Middlebrook and Cohn (3) is used for cultivation of Mycobacteria. This medium can also be used for sensitivity testing of Mycobacteria and for subculturing of stock cultures on addition of Middlebrook OADC Growth Supplement (FD018) and glycerol.

Middlebrook media consists of many inorganic salts, which help, in growth of Mycobacteria. Citric acid formed from sodium citrate helps in retaining inorganic cations in solution. Glycerol supplies carbon and energy. Middlebrook OADC Growth Supplement (FD018) contains oleic acid, bovine albumin, sodium chloride, dextrose and catalase. Oleic acid and other long chain fatty acids are essential for metabolism of Mycobacteria. Some free fatty acids are toxic to Mycobacteria but albumin binds to those fatty acids and prevents toxic action on Mycobacteria. Dextrose serves as an energy source. Catalase neutralizes toxic peroxides. Malachite green partially inhibits other bacteria (1, 4).

Mycobacteria are strict aerobes and therefore increased CO2 tension and aerobic conditions must be provided during incubation. Care should be taken while decontamination of the specimen. Also proper specimen collection (sputum and not saliva) should be ensured. Samples should be carefully handled to avoid contamination.

**Quality Control**

**Appearance**

Light yellow to light green homogeneous free flowing powder
Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Light amber coloured clear to slightly opalescent gel with greenish tinge forms in Petri plates

Reaction
Reaction of 1.97% w/v aqueous solution at 25°C. pH : 6.6±0.2

pH
6.40-6.80

Cultural Response
M197: Cultural characteristics observed with added Middlebrook OADC Growth Supplement (FD018) after an incubation at 35-37°C for 2-4 weeks.

Organism Growth

*Mycobacterium tuberculosis* good-luxuriant
*H37RV* (25618)

*Mycobacterium fortuitum* good-luxuriant
*ATCC 6841*

*Mycobacterium smegmatis* good-luxuriant
*ATCC 14468*

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:
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.