Legionella Agar Base, Granulated

Legionella Agar Base, granulated with the addition of supplement is used for the cultivation of *Legionella* species.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Charcoal activated</td>
<td>2.000</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>10.000</td>
</tr>
<tr>
<td>Agar</td>
<td>13.000</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>6.9±0.2</td>
</tr>
</tbody>
</table>

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

**Directions**

Suspend 12.5 grams in 440 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 and aseptically add contents of 1 vial of Legionella Growth Supplement (BCYE) (FD142). In case of non incorporation of Legionella (GVPC) Selective Supplement (FD143), add aseptically 10 ml sterile distilled water to bring the total volume to 500 ml of medium. The final pH of the medium will be 6.9 ± 0.2. Mix well and pour into sterile petri plates. Stir the medium while dispensing to prevent the settling of charcoal particles. If desired, the medium can be made selective by aseptically adding rehydrated contents of 1 vial of either Legionella BMPA Selective Supplement (FD144) or Legionella (GVPC) Selective Supplement, (FD143) along with 1 vial of Legionella Growth Supplement (BCYE)(FD142) to 440 ml sterile molten, cooled Legionella Agar Base. Simultaneously, a medium without L-Cysteine may be prepared by adding aseptically contents of 1 vial of Legionella Growth Supplement w/o L-Cysteine (FD206).

**Principle And Interpretation**

Legionella Agar initially called as F-G agar was modified by Feely et al (1) by replacing Starch with charcoal and casein hydrolysate with yeast extract which resulted in better recovery of *Legionella pneumophila* (2).

Pasculle et al (3) reported that the addition of ACES (N-2-acetamido-2-amino ethane sulphonic acid) buffer improved the nutritive value of medium. Edelstein (4) suggested addition of a-Ketoglutarate to increase the sensitivity of this medium.

The medium contains yeast extract to provide the necessary nitrogenous nutrients for *Legionella growth*. Activated charcoal nullifies toxic compounds that either accumulate in the medium during growth or develop during sterilization of medium. Addition of ACES buffer helps in maintaining proper pH of the medium for the optimal growth of *Legionella*. Antibiotics in the supplement inhibits the growth of various contaminating bacteria and fungi (4,5).

*Legionella* species have an absolute nutritional requirement for L-Cysteine. Presumptive *Legionella* species colonies can be subcultured onto both Legionella Agar Base with FD142 and with FD206 (Medium without L-Cysteine). All plates are incubated at 35°C. Colonies which grow on Legionella Agar Base with FD142, with L-Cysteine, but not on Legionella Agar Base with FD206 without L-Cysteine, can be regarded as presumptive *Legionella* species.

**Quality Control**

**Appearance**

Grey to black coloured granular medium

**Gelling**

Firm, comparable with 1.3% Agar gel.

**Colour and Clarity of prepared medium**

Black coloured opaque gel forms in petri plates.

**Reaction**

Reaction of 2.5% w/v aqueous solution on addition of Legionella Growth Supplement (FD142) at 25°C. pH : 6.9±0.2

Please refer disclaimer Overleaf.
**Cultural Response**

Cultural characteristics observed with added Sterile Legionella Growth Supplement (BCYE) (FD142) and Legionella (GVPC) Selective Supplement (FD143) or Legionella Growth Supplement w/o L-Cysteine (FD206), after an incubation at 35-37°C for 48-72 hours.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Growth (with FD142 &amp; FD143)</th>
<th>Growth (With FD206)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli ATCC 25922</td>
<td>inhibited</td>
<td>good</td>
</tr>
<tr>
<td>Legionella dumoffii ATCC 33343</td>
<td>good-luxuriant</td>
<td>inhibited</td>
</tr>
<tr>
<td>Legionella pneumophila ATCC 33153</td>
<td>good-luxuriant</td>
<td>inhibited</td>
</tr>
</tbody>
</table>

**Storage and Shelf Life**

Store below 30°C in tightly closed container and 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.