Charcoal Agar

Intended Use
Charcoal Agar is used for cultivating fastidious organisms, especially *Bordetella pertussis*, for vaccine production and stock culture maintenance.

Summary and Explanation
Charcoal Agar is prepared according to the method of Mishulow, Sharpe and Cohen. The authors found this medium to be an efficient substitute for Bordet-Gengou Agar in the production of *B. pertussis* vaccines.

User Quality Control

### Identity Specifications

**Difco™ Charcoal Agar**
- **Dehydrated Appearance**: Gray, free-flowing, homogeneous.
- **Solution**: 6.25% solution, soluble in purified water upon boiling. Solution is black, opaque with a precipitate.
- **Prepared Appearance**: Black, opaque.
- **Reaction of 6.25% Solution at 25°C**: pH 7.3 ± 0.2

### Cultural Response

**Difco™ Charcoal Agar**
Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C under 5-10% CO₂ for 18-72 hours.

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>ATCC®</th>
<th>INOCULUM CFU</th>
<th>RECOVERY</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bordetella bronchiseptica</em></td>
<td>4617</td>
<td>10⁵-10⁶</td>
<td>Good</td>
</tr>
<tr>
<td><em>Bordetella parapertussis</em></td>
<td>15237</td>
<td>10⁵-10⁶</td>
<td>Good</td>
</tr>
<tr>
<td><em>Bordetella pertussis</em></td>
<td>8467</td>
<td>10⁵-10⁶</td>
<td>Good</td>
</tr>
</tbody>
</table>

The genus *Bordetella* consists primarily of four species: *Bordetella pertussis*, *B. parapertussis*, *B. bronchiseptica* and *B. avium*; additional species have recently been described. All *Bordetella* are respiratory pathogens, residing on the mucous membranes of the respiratory tract. *B. pertussis* is the major cause of whooping cough or pertussis. *B. parapertussis* is associated with a milder form of the disease. *B. bronchiseptica* is an opportunistic human pathogen associated with both respiratory and non-respiratory infections, often occurring in patients having close contact with animals. *B. bronchiseptica* has not been reported to cause pertussis. There have been no reports of recovery of *B. avium* from humans.
Charcoal Agar supplemented with Horse Blood is used for the cultivation and isolation of *Haemophilus influenzae*.

**Principles of the Procedure**

Infusion from beef heart and peptone provide the nitrogen, carbon and amino acids in Charcoal Agar. Yeast extract is a vitamin source. Sodium chloride maintains osmotic balance. Agar is the solidifying agent. Soluble starch and Norit SG, charcoal, neutralize substances toxic to *Bordetella* species, such as fatty acids.

**Formula**

**Difco™ Charcoal Agar**

Approximate Formula* Per Liter

- Beef Heart, Infusion from 500 g ................................ 12.0 g
- Peptone .................................................................... 10.0 g
- Sodium Chloride ......................................................... 5.0 g
- Soluble Starch ........................................................... 10.0 g
- Yeast Extract ............................................................... 3.5 g
- Norit SG ...................................................................... 4.0 g
- Agar ......................................................................... 18.0 g

*Adjusted and/or supplemented as required to meet performance criteria.

**Directions for Preparation from Dehydrated Product**

1. Suspend 62.5 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Mix thoroughly during dispensing to uniformly distribute the charcoal.
5. Test samples of the finished product for performance using stable, typical control cultures.

**Procedure**

For a complete discussion on the isolation and maintenance of fastidious microorganisms refer to the procedures described in appropriate references.

**Expected Results**

Refer to appropriate references and procedures for results.

**Limitation of the Procedure**

Charcoal has a tendency to settle out of the medium. Swirl the flask gently when dispensing to obtain a uniform charcoal suspension.

**References**


**Availability**

**Difco™ Charcoal Agar**

Cat. No. 289410  Dehydrated – 500 g