

CHARCOAL AGAR (7586)

Intended Use

Charcoal Agar is used for the cultivation of fastidious microorganisms, particularly *Bordetella pertussis*.

Product Summary and Explanation

Charcoal Agar is prepared according to the method of Mishulow, Sharpe, and Cohen.¹ Charcoal Agar became an efficient substitute for Bordet-Gengou Agar in the production of *Bordetella pertussis* vaccines. This medium is used as a maintenance medium for stock cultures of *Bordetella* spp., and when supplemented with horse blood, for cultivation and isolation of *Haemophilus influenzae*.²

Bordetella consists of four species: *Bordetella pertussis*, *B. parapertussis*, *B. bronchiseptica*, and *B. avium*.³ All *Bordetella* spp. are respiratory pathogens, residing on mucous membranes of the respiratory tract. *B. pertussis* is the major cause of whooping cough or pertussis.

Principles of the Procedure

The nitrogen and carbon sources are provided by Beef Heart Infusion Solids and Enzymatic Digest of Gelatin. Yeast Extract is the vitamin source in this medium. Sodium Chloride maintains the osmotic environment. Soluble Starch and charcoal absorb toxic metabolites.

Formula / Liter

Beef Heart Infusion Solids	12 g
Enzymatic Digest of Gelatin.....	10 g
Sodium Chloride	5 g
Soluble Starch	10 g
Yeast Extract	3.5 g
Charcoal.....	4 g
Agar	18 g

Final pH: 7.3 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 62.5 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. Cool medium to 45 - 50°C and gently remix medium prior to dispensing.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and charcoal grey.

Prepared Appearance: Prepared medium is black and opaque.

Expected Cultural Response: Cultural response on Charcoal Agar were incubated aerobically at 35 ± 2°C and examined for growth after 1 - 4 days.

Microorganism	Approx. Inoculum (CFU)	Expected Results
<i>Bordetella bronchiseptica</i> ATCC® 4617	10 – 300	Growth
<i>Neisseria meningitidis</i> ATCC® 13090	10 - 300	Growth
<i>Streptococcus pneumoniae</i> ATCC® 6305	10 - 300	Growth
<i>Streptococcus pyogenes</i> ATCC® 19615	10 - 300	Growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for a complete discussion on isolation, identification, and maintenance of *Bordetella* spp. and other fastidious microorganisms.^{3,4}

Results

Refer to appropriate references for results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. Charcoal has a tendency to settle out of the medium. Swirl the flask gently when dispensing to obtain a uniform charcoal suspension.²

Packaging

Charcoal Agar	Code No.	7586A	500 g
		7586B	2 kg
		7586C	10 kg

References

1. **Mishulow, L., L. S. Sharpe, and L. L. Cohen.** 1953. Beef-heart charcoal agar for the preparation of pertussis vaccines. *Am. J. Public Health*, **43**:1466.
2. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, p. 110-114. Williams & Wilkins, Baltimore, MD.
3. **Marcon, M. J.** 1995. *Bordetella*, p. 566-573. In P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.), *Manual of clinical microbiology*, 6th ed. American Society for Microbiology, Washington, D.C.
4. **Isenberg, H. D. (ed.).** 1992. *Clinical microbiology procedures handbook*. American Society for Microbiology, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (517)372-9200 or fax us at (517)372-2006.