

Charcoal Agar Base

Used for cultivation of *Bordetella pertussis*, for vaccine production and also for stock culture maintenance.

OREF: BS.1/NZ01.100.0100	100 Gram	REF: BS.1/NZ01.250.0250	250 Gram
REF: BS.1/NZ01.500.0500	500 Gram		



MEDIA COMPOSITION

PRINCIPLE AND INTERPERTATION :

The genus *Bordetella* comprises four species: *Bordetella pertussis*, *Bordetella parapertussis*, *Bordetella bronchiseptica* and *Bordetella avium* (1). Genetic studies have demonstrated that these organisms are closely related. *B. pertussis* and *B. parapertussis* are exclusively human pathogens, whereas *B. bronchiseptica* infects a wide variety of animals and is only occasionally isolated from humans (2). *B. avium* primarily infects birds. Members of the genus are obligately aerobic and metabolically inactive, with *B. bronchiseptica* being the only motile species. *B. pertussis* is the principal causative agent of whooping cough (pertussis), while *B. parapertussis* is associated with a milder form of the disease (3). Primary isolation of *B. pertussis* requires an enriched medium containing charcoal and 15–20% blood to neutralize growth-inhibitory substances and support its recovery. Charcoal Agar, prepared according to the method of Mishulow, Sharpe and Cohen (2), serves as an effective alternative to Bordet-Gengou Agar for the isolation of *B. pertussis* and for the production of *B. pertussis* vaccines. When supplemented with horse blood, Charcoal Agar can also be used for the cultivation and isolation of *Haemophilus influenzae* (4). Isolation of *B. pertussis* from nasopharyngeal secretions is often complicated by the overgrowth of normal flora during the prolonged incubation period required on enriched media. Penicillin may be incorporated into the medium to suppress contaminating microorganisms; however, penicillin-resistant flora may still interfere with isolation, as reported by Lacey (4). Broome et al. demonstrated that methicillin was more effective than penicillin in suppressing unwanted nasopharyngeal flora (5), while Sutcliffe and Abbott reported that cephalexin provided even greater selectivity than methicillin (6). HM Infusion B, peptone and yeast extract provide essential nutrients required for the growth of *Bordetella* species. Sodium chloride maintains the osmotic balance of the medium, while soluble starch and activated charcoal adsorb toxic substances, particularly fatty acids, that may inhibit bacterial growth. Since charcoal readily settles at the bottom of the container, the medium should be gently swirled before dispensing to ensure a uniform suspension (7). After incubation, plates should be examined after approximately 40 hours and subsequently twice daily. Typical *Bordetella* colonies appear as small, smooth, shiny, grayish-white, rounded colonies. Presumptive isolates should be confirmed by Direct Fluorescent Antibody (DFA) testing. For earlier diagnosis, DFA testing may also be performed directly on nasopharyngeal secretions.

Item	Formula per liter of medium g/l
Peptone	10.0
Yeast extract	3.50
Starch, soluble	10.0
Charcoal	4.0
Sodium chloride	5.0
HM infusion B from 500g#	12.0
Agar	18.0

Final pH 7.0 ± 0.2

** Formula adjusted, standardized to suit performance parameters.

#Equivalent to beef heart, infusion from

PRECAUTIONS AND WARNINGS

Media to be handled by entitled and professionally educated person. Do not ingest or inhale.

Good Laboratories practices using appropriate precautions should be followed in:

- Wearing personnel protective equipment (overall, gloves, glasses.).
- Do not pipette by mouth.
- In case of contact with eyes or skin; rinse immediately with plenty

of soap and water. In case of severe injuries; seek medical advice immediately.

- Respect country requirement for waste disposal.
- S56:** dispose of this material and its container at hazardous or

special waste collection point.

S57: use appropriate container to avoid environmental contamination.

S61: avoid release in environment.

For further information, refer to the Charcoal Agar Base material

safety data sheet.

STORAGE AND STABILITY

BioScien Charcoal Agar Base Store between 10-30°C in a tightly closed container and the prepared medium at 15-25°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use Product performance is best if used within stated expiry period.

PREPARATION

Suspend 31.25 grams in 450 ml purified/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° and aseptically add sterile 10% of defibrinated blood and rehydrated contents of one vial of Bos Selective Supplement (FD004). Mix well and pour into sterile Petri plates. For *Haemophilus* species, the medium can be converted to Chocolate Agar.

Deterioration

The color of **BioScien** Charcoal Agar Base is Grey to greyish black homogeneous free flowing powder. Colour and Clarity of prepared medium Black coloured, opaque gel with undissolved black particles forms in Petri plates.

EQUIPMENT REQUIRED NOT PROVIDED

- Sterile petri dishes
- Incubator
- Autoclave

PERFORMANCE CHARACTERISTICS

Cultural characteristics observed with added sterile defibrinated blood and Bos Selective Supplement (FD004), after an incubation at 35 - 37°C for 24 - 48 hours

Organism	Growth
<i>Bordetella bronchiseptica</i> ATCC 4617	Good -Luxuriant
<i>Bordetella parapertussis</i> ATCC 15311	Good -Luxuriant
<i>Bordetella pertussis</i> ATCC 8467	Good -Luxuriant
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 25923	inhibited
<i>Klebsiella pneumoniae</i> ATCC 13883	inhibited

QUALITY CONTROL

To ensure adequate quality control, it is recommended that positive and negative control included in each run. If control still out of range please contact **BioScien** technical support.

REFERENCE

1. Murray P. R., Baron J. H., Tenover F. C., Tenover J. C., Jorgensen J. H. and Tenover J. C., (Ed.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.
2. Mishulow, Sharpe and Cohen, 1953, Am. J. Public Health, 43:1466.
3. Ensminger P. W., Gulberston C. G. and Powell H. M., 1953. J. Infect. Dis., 93(3):266.
4. Lacey B. W., 1954, J. Hyg., 59:273

5. Broome C. V., Fraser D. W. and English J. W., 1979, Internat. Symp. on Pertussis DHEW J., Washington D.C., pp 19-29.
6. Sutcliffe E. M. and Abbott J. D., 1979, B.M.J. II: 732-733.
7. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.
8. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
9. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

SYMBOLS IN PRODUCT LABELLING			
	For in-vitro diagnostic use		Number of <n> test in the pack
	Batch Code/Lot number		Caution
	Catalogue Number		Do not use if package is damaged
	Temperature Limitation		Consult Instruction for use
	Expiration Date		
	Manufactured by		