



## Instructions for Use

# CHOCOLATE AGAR WITH BACITRACIN

[Cat. no. E11](#)

Chocolate with Bacitracin, 15x100mm Plate, 18ml

10 plates/bag

## INTENDED USE

Hardy Diagnostics Chocolate Agar with Bacitracin is recommended for use in the isolation and cultivation of *Haemophilus* species from respiratory specimens containing mixed flora.

## SUMMARY

Chocolate Agar with Bacitracin consists of GC Agar Base with added hemoglobin and KoEnzyme Enrichment. GC Agar Base contains proteose peptone which provides nitrogenous nutrients. When heated, hemoglobin releases hemin (X-factor), a factor required by fastidious organisms such as *Haemophilus* species. The phosphate buffer is added to maintain the pH of the medium. Corn starch aids in neutralizing any toxic fatty acids present in the medium. KoEnzyme Enrichment is a chemically defined enrichment that provides NAD (V-factor), amino acids, vitamins, dextrose, ferric ions, and coenzymes which promote growth. Bacitracin inhibits most strains of streptococci, staphylococci, *Neisseria* and *Micrococcus* species. Because *Haemophilus* spp. are resistant to the concentration of bacitracin employed in this medium, Chocolate Agar with Bacitracin allows for their increased recovery, due to the decreased competition for nutrients in the medium.

## FORMULA

Ingredients per liter of deionized water:\*

<b>Chocolate Agar with Bacitracin:</b>	
Proteose Peptone	15.0gm
Sodium Chloride	5.0gm
Dipotassium Phosphate	4.0gm
Monopotassium Phosphate	1.0gm
Corn Starch	1.0gm
Hemoglobin, Bovine	10.0gm
KoEnzyme Enrichment	10.0ml
Bacitracin	20,000U
Agar	10.0gm
<b>KoEnzyme Enrichments:</b>	

Dextrose	10.0gm
L-Cystine HCl	2.59gm
L-Glutamine	1.01gm
L-Cystine	0.11gm
Adenine	0.101gm
Nicotinic Adenine Dinucleotide	25.0mg
Coccarboxylase	10.0mg
Guanine Hydrochloride	3.0mg
Ferric Nitrate	2.0mg
P-Aminobenzoic Acid	1.3mg
Vitamin B <sub>12</sub>	1.0mg
Thiamine	0.3mg

Final pH 7.2 +/- 0.2 at 25°C.

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

## STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8°C. away from direct light. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

The expiration dating on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended quality control incubation times.

Refer to the document "[Storage](#)" for more information.

## PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual universal blood precautions. Do not ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." The "Guidelines for Isolation Precautions" is available from the Centers for Disease Control and Prevention at [www.cdc.gov/ncidod/dhqp/gl\\_isolation.html](http://www.cdc.gov/ncidod/dhqp/gl_isolation.html).

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M-29: *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline*.

Sterilize all biohazard waste before disposal.

Refer to the document "[Precautions When Using Media](#)" for more information.

Refer to the document [SDS Search](#) instructions on the Hardy Diagnostics' website for more information.

## PROCEDURE

**Specimen Collection:** Consult listed references for information on specimen collection. <sup>(1-3,5,7)</sup> Infectious material should be submitted directly to the laboratory without delay and protected from excessive heat and cold. If there is to be a delay in processing, the specimen should be inoculated onto an appropriate transport media and maintained at an appropriate temperature. <sup>(1-3,5,7)</sup>

**Method of Use:** Inoculate the medium and streak the specimen as soon as possible after it is received in the clinical laboratory. If the specimen is being cultured directly from a swab, roll the swab over a small portion of the agar surface, and streak for isolation. Incubate in 5-10% CO<sub>2</sub> at 35-37°C. for 24 to 48 hours. Extend incubation if needed. Examine plate for typical colonial morphology and characteristics.

## INTERPRETATION OF RESULTS

Consult listed references for the interpretation of growth of fastidious species. <sup>(1-3,5,7)</sup>

## LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification.

*E. coli*, some *Neisseria* species, strains of *Candida* spp., *Klebsiella*, *Proteus*, and *Pseudomonas* spp., as well as other bacteria may grow on this medium.

Refer to the document "[Limitations of Procedures and Warranty](#)" for more information.

## MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, other culture media, swabs, applicator sticks, incinerators, and incubators, etc., as well as serological and biochemical reagents, are not provided.

## QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificates of Analysis (CofA). The following organisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
<i>Haemophilus influenzae</i> ATCC <sup>®</sup> 10211	A	24hr	35°C	CO <sub>2</sub> **	Growth
<i>Staphylococcus epidermidis</i> ATCC <sup>®</sup> 12228	B	24hr	35°C	Aerobic	Inhibited

\* Refer to the document "[Inoculation Procedures for Media QC](#)" for more information.

## USER QUALITY CONTROL

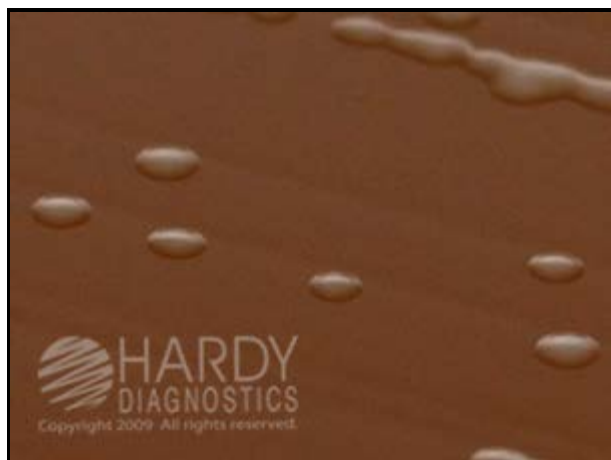
End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures

or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificates of analysis (CofA) available from Hardy Diagnostics [Certificates of Analysis](#) website. In addition, refer to the following document "[Finished Product Quality Control Procedures](#)," for more information on QC or see reference(s) for more specific information.

\*\* Atmosphere of incubation is enriched with 5-10% CO<sub>2</sub>.

## PHYSICAL APPEARANCE

Chocolate Agar with Bacitracin should appear opaque, and brown in color.



*Haemophilus influenzae* (ATCC<sup>®</sup> 10211) colonies growing on Chocolate Agar with Bacitracin (Cat. no. E11). Incubated in CO<sub>2</sub> for 24 hours at 35°C.



*Staphylococcus epidermidis* (ATCC<sup>®</sup> 12228) growth inhibited on Chocolate Agar with Bacitracin (Cat. no. E11). Incubated aerobically for 24 hours at 35°C.

## REFERENCES

1. Anderson, N.L., et al. *Cumitech 3B; Quality Systems in the Clinical Microbiology Laboratory*, Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.
2. Jorgensen., et al. *Manual of Clinical Microbiology*, American Society for Microbiology, Washington, D.C.
3. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology*, C.V. Mosby Company, St. Louis, MO.
4. Carpenter, C.M. and H.E. Morton. 1947. Proc. N.Y. State Assoc. Public Health Labs, 27:58-60.
5. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I, II & III. American Society for Microbiology, Washington, D.C.
6. Johnston, et al. 1945. *J. Ven. Dis. Inf.*; 26:239.
7. Koneman, E.W., et al. *Color Atlas and Textbook of Diagnostic Microbiology*, J.B. Lippincott Company, Philadelphia, PA.
8. Maritn, J.E., et al. 1967. *Public Health Rep.*; 82:361.
9. McLeod, J.W., et al. 1927. *Br. J. Exp. Pathol.*; 8:25.
10. *Quality Assurance for Commercially Prepared Microbiological Culture Media*, M22-A2, Vol. 16, No.16. 1996. Clinical Laboratory Standards Institute (CLSI - formerly NCCLS), Villanova, PA.

ATCC is a registered trademark of the American Type Culture Collection.

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