CHOCOLATE AGAR (ENRICHED)

- For in vitro use only - Catalogue No. TC55

Our Chocolate Agar (Enriched) is used for the isolation and cultivation of fastidious microorganisms such as *Neisseria* and *Haemophilus* species.

The first formulation of chocolate agar was devised in 1927 by McLeod, et al that contained a combination of yeast extract and various peptones. Modifications were later made by Johnston and Martins et al. that helped improve the recovery of *Neisseria* species and to shorten the incubation time from 48 hours to 24 hours.

Our formulation contains an improved casein and animal tissue digest that provide the organism with nitrogen, amino acids, and other elements essential for growth. Neisseria species are highly sensitive to toxic substances such as fatty acids; therefore the addition of cornstarch helps neutralize possible toxic metabolites, while potassium phosphate helps maintain an uniform pH during growth. Hemoglobin (Cat No. VH55) provides X-factor (hemin) required by some *Haemophilus* species, and Isovitox Enrichment (Cat No. VI85) provides V-factor (nicotinamide dinucleotide), cocarboxylase and other complex compounds that enhance the growth of *Neisseria* species.

Formula per Litre of Medium

Casein/Animal Tissue Digest	15.0 g
Cornstarch	1.0 g
Potassium Phosphate, Dibasic	4.0 g
Potassium Phosphate, Monobasic	1.0 g
Sodium Chloride	5.0 g
Agar	10.0 g
Hemoglobin Solution (2%)	500 0 mL
Isovitox Enrichment	10.0 mL

pH 7.2 ± 0.2

Recommended Procedure

1. Allow medium to reach room temperature.
2. Using an inoculum from the specimen, streak the slant in a fish tail motion from the bottom to the top.
3. Cap tubes loosely to allow for gas exchange.
4. Incubate in a 5 to 10% CO_2 atmosphere at 35°C.
5. Examine after 24 hours and again at 48 hours.

Interpretation of Results

Chocolate Agar is an enriched general-purpose medium that supports the growth of most fastidious and non-fastidious organisms. Because it is a non-selective medium, resident flora from clinical specimens may overgrow potential fastidious pathogens, such as *Neisseria* species.

Neisseria gonorrhoeae produces small, grey to white, mucoid colonies. *N. meningitidis* produces larger bluish-grey, mucoid colonies.

Haemophilus influenzae produces small, colorless, moist colonies with a characteristic "mousy" odour.

Additional biochemical and/or serological tests should be performed on isolated colonies from pure culture in order to complete identification.

- *Chocolate Agar contains less agar than other solid media therefore streaking should be done carefully to avoid gouging into the agar*

Quality Control

After checking for correct pH, colour, depth, and sterility, the following organisms are used to determine the growth performance of the completed medium.

Organism	Expected Result
Neisseria gonorrhoeae ATCC 43069	Growth
Haemophilus influenzae ATCC 10211	Growth

Storage and Shelf Life

Our Chocolate Agar (Enriched) should be stored away from direct light at 4 to 8°C in an upright position. Under these conditions this medium has a shelf life of 10 weeks from the date of manufacture.

Ordering Information

Cat#	Description	Format
TC55-05	Chocolate Agar Slant 5-mL [16x125-mm s/c tube]	10/pkg
TC55-16	Chocolate Agar Slant 16-mL [20x150-mm s/c tube]	10/pkg

References

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3. Lankford CE, Snell EE. Glutamine as a growth factor for certain strains of *Neisseria gonorrhoeae*. J Bacteriol 1943; 45:421.
4. Johnston J. Comparison of gonococcus cultures read at 24 and 48 hours. J Verera Dis Inform 1945; 26:239.
5. Carpenter CM, Bucca MA, Buck TC, Casman EP, Christensen CW, Crowe E, Drew R, Hill J, Lankford CE, Morton HE, Peizer LR, Shaw I, Thayer JD. Am J Syphil Gonnorh Vener Dis 1949; 33:164.
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7. MacFaddin JF. Media for isolation-cultivation-identification-maintenance of medical bacteria, Vol I. Baltimore, MD: Williams & Wilkins, 1985.
8. Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH. Manual of clinical microbiology. 7th ed. Washington D.C.: ASM, 1999.

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