



CHOCOLATE AGAR (ENRICHED)

- For in vitro use only -

Catalogue No. PC55

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 a uniform pH during growth. Hemoglobin (Dalynn VH55) provides X-factor (hemin) required by *Haemophilus* species, and isovitox enrichment (Dalynn No. VI85) provides V-factor (nicotinamide dinucleotide), cocarboxylase and other complex compounds that enhance the growth of *Neisseria* species.

Formula per Litre of Medium

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|--------------------------------------|----------|
| 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, perform a four-quadrant streak to obtain well-isolated colonies.
3. Incubate in a 5 to 10% CO₂ atmosphere at 35°C.
4. Examine after 18-24 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. If contaminating bacteria are of concern the sample can be streaked out onto a selective chocolate medium, such as our Modified Martin-Lewis Agar (Catalogue No. PC60).

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.

