CHOCOLATE AGAR WITH BIOVITEX

Intended Use

Chocolate Agar is used with hemoglobin and enrichment for the isolation and cultivation of *Haemophilus influenzae* and other fastidious organisms.

Principles of the Procedure

Enzymatic Digest of Casein and Enzymatic Digest of Animal Tissue provide nitrogen, carbon, and minerals in GC Agar. Corn Starch absorbs any toxic metabolites produced. The Phosphates are buffering agents. Sodium Chloride maintains osmotic balance of the medium. Agar is the solidifying agent. Chocolate Agar is prepared from GC Agar with the addition of Hemoglobin. Hemoglobin provides hemin (X factor) required for growth of *Haemophilus* and enhanced growth of *Neisseria* spp. A chemical enrichment composed of cofactors, vitamins, and nicotinamide adenine dinucleotide (NAD) are also required for growth of *Haemophilus* and *Neisseria* spp. If required, antimicrobial supplements are added as inhibitors for improved selectivity of the medium.

Formula / Liter

Enzymatic Digest of Casein..... 7.5 g
Enzymatic Digest of Animal Tissue..... 7.5 g
Corn Starch..... 1.0 g
Dipotassium Phosphate..... 4.0 g
Monopotassium Phosphate..... 1.0 g
Sodium Chloride..... 5.0 g
Agar..... 10.0 g

Supplements:

Hemoglobin Powder, 10 g
Growth Enrichment, 2 mL

Final pH: 7.2 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.

Directions

1. Suspend 36,0 g of the GC Agar in 500 mL 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. Cool to 45 – 50°C.
4. Suspend 10,0 g of hemoglobin powder in 500 mL of purified water and autoclave at 121°C for 15 minutes.
5. Cool to 45 - 50°C and aseptically add to the molten GC Agar. Add 2 mL of growth enrichment. Mix thoroughly and dispense.

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.
Prepared Appearance: Prepared Chocolate Agar is opaque and brown.
Expected Cultural Response: Cultural response on Chocolate Agar at 35°C under CO_2 enrichment after 18 – 24 hours incubation.

Microorganism	Response
Haemophilus influenza ATCC® 10211	growth
Neisseria gonorrhoeae ATCC® 43070	growth
Neisseria meningitidis ATCC® 13090	growth
Streptococcus agalactiae ATCC® 13813	growth
Streptococcus pneumoniae ATCC® 6303	growth

Test Procedure

For a complete discussion on the isolation and identification of *Neisseria* spp. and *Haemophilus* spp. consult procedures outlined in the references.[8,9]

Results

Refer to appropriate references and procedures for results.

Storage ready to use plates - 6-12°C

Packaging cat No. 1080 ready to use plates Ø 90 mm (1 x 10 pcs);

Expiration ready to use plates - 90 days

Limitation of the Procedure

Although certain diagnostic tests may be performed directly on Chocolate Agar, biochemical and immunological testing using pure cultures are recommended for complete identification.

References

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7. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, Williams & Wilkins, Baltimore, MD.
8. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook. vol. 1. American Society for Microbiology, Washington, D.C.
9. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (eds.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Graso Biotech
Kr□ g 4A, 83-200 Starogard Gda□ ski
Customer service: 58 562 30 21, fax. 58 562 79 87,
zamowienia@graso.com.pl; www.podloza.pl, www.grasobiotech.pl