HAEMOPHILUS CHOCOLATE AGAR

INSTRUCTION FOR USE READY-TO-USE PLATED MEDIA

For professional use

Intended use: Haemophilus Chocolate Agar is used for the isolation and cultivation of Haemophilus influenzae.

Ref.:	Type of medium:	Packaging:
201261	ready-to-use medium-plate	1x20 pcs (90 mm)

1. Principle: enzymatic digest of casein and enzymatic digest of animal tissue provide nitrogen, carbon, and minerals. 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. Hemoglobin provides hemin (X factor) required for growth of *Haemophilus* spp. A chemical enrichment composed of cofactors, vitamins, and NAD (Biovitex) are also required for growth of *Haemophilus* spp. Vancomycin, amphotericin, bacitracin are selective agents which improved selectivity of the medium.

2. Formula/Liter:

Enzymatic digest of casein	7.5 g
Enzymatic digest of animal tissue	7.5 g
Corn starch	1.0 g
Agar	10.0 g
Sodium chloride	5.0 g
Monopotassium Phosphate	1.0 g
Dipotassium phosphate	4.0 g

Supplements/Liter:

Hemoglobin	10.0 g
Vancomycin	0.004 g
Amphotericin	0.004 g
Bacitracin	16500 IU
Biovitex	10.0 ml

3. pH: 7.2 ± 0.2 at 25°C.

4. Appearance:

Prepared Appearance: prepared medium is brown and homogenous.

5. Sample: all samples in which a *Haemophilus* spp. is expected.

6. Test procedure: if the agar plate has been refrigerated, allow to warm to room temperature before inoculation. Streak the specimen for isolation onto the surface of the medium. If the specimen is cultured from a swab, roll the swab gently over a small area of the surface at the edge, then streak from this area with a sterile loop. Incubate plates aerobically at 35±2°C for 18 - 24 hours in an inverted position.

7. Results: after incubation observe characteristic growth of microorganism. Identification of the microorganism should be confirmed by biochemical test.

8. Quality control: perform quality control testing for both negative and positive reaction by inoculating a representative sample of plates with pure cultures of stable control organisms that produce known, desired reactions. Graso uses following strains for performing quality control. Please note that other strains can be used in accordance with applicable local, state and laboratory's standard Quality Control.

Microorganism:	Appearance of colony:
Haemophilus influenzae ATCC 49766	small, mucoid, pearl
Escherichia coli ATCC 25922	large, flat, grey
Candida albicans ATTC 10231	-
Staphylococcus aureus ATCC 25923	-

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

10. Disposal of waste: after use, all plates and any other contaminated materials must be sterilized or disposed of in line with appropriate internal procedures and in accordance with local legislations. Plates can be destroyed by autoclaving at 121°C for at least 20 minutes.

11. Storage: on receipt, store plates at 2-12°C away from direct sun light in an inverted position. Do not overload a refrigerator with excessive amounts of plates to avoid water condensation on the lids during storage. Plates must not come into direct contact with the inner walls of refrigerator, as the media may freeze, invalidating the tests. Prepared plates, stored in their original sleeve wrapping at 2-12°C until just prior to use, may be inoculated up to the expiration date and incubated for recommended incubation times. Plates from opened stacks of 10 plates should be used for two weeks when stored in a clean area at 2 to 12° C. Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or others signs of deterioration. Allow the medium to warm to the room temperature before inoculation.

All microbiological media containing dyes or light-sensitive components should be protected from light and stored in the dark.

Note that shelf life of the growth media changes after the addition of supplements. Complete media containing protein supplement tend to degrade faster than basal media alone.

12. Shelf life: 3 months.

13. Required supplements not supplied together with medium base: not applicable.

14. References: available on request.

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