

Chocolate Agar

Catalog : HB8641-2

Chocolate Agar is used for the isolation of *Neisseria gonorrhoeae* from chronic and acute cases of gonococcal infections.

Approximate Formula:

Ingredients	gm/liter
Pancreatic Digest of Casein	10.0
Peptic Digest of Animal Tissue	5.0
Beef Heart Infusion Solids	3.0
Yeast Extract	5.0
Corn Starch	1.0
Sodium Chloride	5.0
Agar	12.0

Final pH7.3 ± 0.2 at 25 °C

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

Directions:

Suspend 44.0g in 1 litre of distilled water. Heat with frequent agitation until the Mix well and heat with frequent agitation to completely dissolve the powder. Dispense into 200mL flasks, sterilize in an autoclave at 121 °C (15 lbs) for 15 minutes. When cool to about 50 °C, add 7% sterile defibrated sheep blood (about 14mL) to each bottle, mix well, and heat in an 80 °C water bath for about 10 minutes (with frequent shaking), until the color of the culture medium turns brown (chocolate color). Pour into petri dishes.

Principle and Interpretation:

Peptones prepared from pancreatic digest of casein, peptic digest of animal tissue and beef heart infusion solids provide sources of carbon, nitrogen, vitamins and minerals.. Yeast extract and corn starch are also included in the formulation and serve as energy sources with yeast extract being a supplier of the B-complex vitamins. Sodium chloride maintains osmotic balance in the medium. Sheep blood allows detection of hemolytic reactions and supplies the X factor (heme) necessary for the growth of many bacterial species Agar is the solidifying agent.

Appearance:

Dehydrated medium is a free-flowing yellowish powder. The prepared medium is a kind of yellowish transparent gel.

Precautions:

This medium is for laboratory use only. Dried medium which is caking or color variation cannot be used.

Storage conditions and Shelf life:

Chocolate Agar must be stored tightly capped in the original container at 5-30 °C. The dehydrated medium has a shelf life of 3 years from date of manufacturing. Prepared medium may be stored, out of direct light at 2-8 °C.

Quality control:

Prepare the culture medium as per label directions (with 5%-10% sheep blood). Inoculate and incubate at 36 ± 1 °C for 24-48h.

Microorganism	Strains Number	Inoculum (CFU)	Growth	Remarks
<i>Bacillus cereus</i>	ATCC 11778	10-100	luxuriant	β-Hemolytic ring around the Colonies
<i>Staphylococcus Aureus</i>	ATCC 6538	10-100	luxuriant	β-Hemolytic ring around the Colonies
<i>Beta-hemolytic Streptococcus</i>	CMCC 32210	10-100	luxuriant	β-Hemolytic ring around the Colonies
<i>Streptococcus pneumoniae</i>	ATCC 6305	10-100	luxuriant	α-Hemolytic ring around the Colonies

Prepare the culture medium as per label directions (without sheep blood). Inoculate and incubate at 30-35 °C for 24-48 hours in an anaerobic atmosphere.

Microorganism	Strains Number	Inoculum (CFU)	Growth	Remarks
<i>Clostridium sporogenes</i>	ATCC 19404	10-100	luxuriant	Colorless-yellowish colonies
<i>Clostridium perfringens</i>	ATCC 13124	10-100	luxuriant	Colorless-yellowish colonies

References:

- United States Pharmacopeial Convention, Inc. 2008. The United States pharmacopeia 31/The national formulary 26, Supp. 1, 8-1-08, online. United States Pharmacopeial Convention, Inc., Rockville, Md.
- European Directorate for the Quality of Medicines and Healthcare. 2008. The European pharmacopoeia, 6th ed., Supp. 1, 4-1-2008, online. European Directorate for the Quality of Medicines and Healthcare, Council of Europe, 226 Avenue de Colmar BP907-, F-67029 Strasbourg Cedex 1, France.