

CHOCOLATE AGAR BASE

A highly nutritious medium for the isolation and cultivation of fastidious micro-organisms especially *Neisseria* and *Haemophilus* spp.

Dehydrated media	
Code number:	500 g: CHO20500-SE, 5 kg: CHO25000-SE
Colour:	Yellowish
Appearance:	Homogeneous hygroscopic powder
pH before autoclaving (25 °C):	7,0 – 7,4

Direction for Chocolate Agar: Suspend **20 g** in 460 ml of distilled water and heat with frequent agitation until the medium boils well. Sterilise by autoclaving at 121 °C for 15 minutes. Cool to 50 °C and add aseptically **35 ml of sterile defibrinated blood** and “chocolate” by heating at 80 °C for 10 min. Cool to 50 °C Dissolve the contents of **one vial of Growth Factor Mixture Hydration Fluid** with 5 ml of sterile distilled water and add aseptically to the **Growth Factor Mixture (GFM80005)**. Mix well and add aseptically to the medium. Mix well before pouring.

Direction for Chocolate Agar + Bacitracin: Dissolve the contents of **one vial of Bacitracin (150 mg) Supplement (BAC80004)** with 4 ml of sterile distilled water and add aseptically to 500 ml of Chocolate Agar at 50 °C. Mix well before pouring.

Direction for Chocolate Agar + Vancomycin: Dissolve the contents of **one vial of Vancomycin (13 mg) Supplement (VSS80004-13)** with 4 ml of sterile distilled water and add aseptically to 500 ml of Chocolate Agar at 50 °C. Mix well before pouring.

Prepared media	
Bottled media bases:	100 ml: CHO30100-SE, 500 ml: CHO30500-SE
Plated Chocolate Agar:	55 mm: CHO50055-02, 90 mm: CHO50090-02
Plated Chocolate Agar + Bacitracin:	55 mm: CHO50055-03, 90 mm: CHO50090-03
Plated Chocolate Agar + Vancomycin:	55 mm: CHO50055-04, 90 mm: CHO50090-04
Appearance of bottled media bases:	Yellowish, transparent gel
Appearance of plated media:	Chocolate brown, homogeneous turbid gel
pH (25 °C):	7,1 – 7,3

Direction: Supplement the melted bottled media bases according to the direction of the dehydrated media and dispense aseptically into sterile Petri-dishes. Media in Petri-dishes are ready to use.

FORMULA in g/l

Nutrient mixture (peptones, extracts)	20
Sodium chloride	5
Buffers	1
Agar	14

Note: The typical formula can be adjusted to obtain optimal performance.

Storage conditions: Store the dehydrated media tightly closed in a dry place at room temperature. Store the bottled media protected from light at room temperature. Store the plated media protected from light at 2-8 °C. Use before the expiry date on the label.

Quality control:

Test strains	Incubation temp: 37 °C	Growth	Incubation time: 24 h
<i>Haemophilus influenzae</i> ATCC 49766		Good	
<i>Streptococcus pyogenes</i> ATCC 19615		Inhibited (in case of selective media)	

References: Carpenter and Morton (1947) Proc. N. Y. State Assoc. Public Health Labs. 27: 58.

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