CHOCOLATE AGAR

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, 5 kg: CHO25000
Packaging of 500 g:	500 g agar base + 25 vials Growth Factor Mixture
Packaging of 5 kg:	5 kg agar base + 250 vials Growth Factor Mixture
Appearance of agar base:	Yellowish, homogeneous hygroscopic powder
Appearance of supplement:	Pink, homogeneous lyophilisate
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 and add aseptically the contents of **one vial of Growth Factor Mixture** reconstituted with 4 ml sterile distilled water. 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.

Bottled media	
Code number:	100 ml: CHO30100, 500 ml: CHO30500
Packaging of 100 ml bottled media:	100 ml agar base + 1 vial Growth Factor Mixture
Packaging of 500 ml bottled media:	500 ml agar base + 1 vial Growth Factor Mixture
Appearance of agar base:	Yellowish, transparent gel
Appearance of supplement:	Pink, homogeneous lyophilisate
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.

Plated media	
Code number of chocolate agar:	55 mm: CHO50055-02, 90 mm: CHO50090-02
Code number of chocolate agar + Bacitracin:	55 mm: CHO50055-03, 90 mm: CHO50090-03
Code number of chocolate agar + Vancomycin:	55 mm: CHO50055-04, 90 mm: CHO50090-04
Appearance of plated media:	Chocolate brown, homogeneous turbid gel
pH (25 °C):	7,1 – 7,3

Direction: Media in Petri-dishes are ready to use.

FORMULA FOR ONE LITRE OF COMPLETE MEDIUM

Nutrient substrate (peptones, extracts)	20,00000 g
L-Cysteine	0,50000 g
L-Glutamine	0,20000 g
L-Cystine	0,02000 g
Glucose	2,00000 g
Sodium chloride	5,00000 g
Ferric nitrate	0,00040 g
Adenine	0,02000 g
NAD	0,00500 g
Cocarboxylase	0,00200 g
Guanine	0,00060 g
p-Aminobenzoic acid	0,00025 g
Thiamine	0,00005 g
Buffers	1,00000 g
Agar	14,00000 g
Sterile defibrinated “chocolated” blood	70 ml

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 supplements and 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
Haemophilus influenzae ATCC 49766		Good	
Streptococcus pyogenes 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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