MUELLER-HINTON II AGAR

An antimicrobial susceptibility testing medium which fits the requirements of EUCAST and CLSI. The medium has extremely low concentrations of thymine and thymidine as well as appropriate levels of calcium and magnesium ions.

Dehydrated media	
Code number:	500 g: MHT20500, 5 kg: MHT25000
Colour:	Yellowish
Appearance:	Homogeneous hygroscopic powder
pH before autoclaving (25 °C):	7,1 – 7,5

Direction for Mueller-Hinton II Agar: Suspend **38 g** in one litre of distilled water and heat with frequent agitation until the medium boils well. Sterilise by autoclaving at 121 °C for 15 minutes.

Direction for Mueller-Hinton II Blood Agar, EUCAST: Suspend **38 g** in one litre 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 **50 ml of sterile defibrinated horse blood and 0,02 g β-NAD**. Mix well before pouring.

Direction for Mueller-Hinton II Chocolate Agar: Suspend **19 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.

Prepared media	
Bottled media:	100 ml: MHT30100, 500 ml: MHT30500
Plated Mueller-Hinton II Agar:	90 mm Petri-dish, 25 ml: MHT50090-01
Plated Mueller-Hinton II Blood Agar, EUCAST:	90 mm Petri-dish, 25 ml: MHT50090-04
Plated Mueller-Hinton II Chocolate Agar:	90 mm Petri-dish, 25 ml: MHT50090-03
Colour of blood free agar:	Yellowish
Colour of blood agar:	Ruby red
Colour of chocolate agar:	Chocolate brown
pH (25 °C):	7,2 - 7,4

Direction: If necessary, blood may be added to the melted bottled media according to the direction of the dehydrated media. Dispense aseptically into sterile Petri-dishes. Media in Petri-dishes are ready to use.

FORMULA in g/l

Acid hydrolisate of casein	17,5
Beef extract	2,0
Starch soluble	1,5
Agar	17,0

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:

Conditions, Mueller-Hinton II Agar:			
Incubation temperature:	35 °C	Incubation time:	16 h
Test strains		**Growth**	**Zone diameter**
Escherichia coli **ATCC25922**		Good	
Ampicillin	10 µg		15 – 22 mm
Gentamicin	10 µg		19 – 26 mm
Tigecycline	15 µg		20 – 27 mm
Trimeth.-Sulfam.	1,25/23,75 µg		23 – 29 mm
Enterococcus faecalis **ATCC29212**		Good	
Trimeth.-Sulfam.	1,25/23,75 µg		26 – 34 mm
Pseudomonas aeruginosa **ATCC27853**		Good	
Gentamicin	10 µg		17 – 23 mm
Tobramycin	10 µg		20 – 26 mm
Staphylococcus aureus **ATCC29213**		Good	
Oxacillin	1 µg		19 – 25 mm
Gentamicin	10 µg		19 – 25 mm
Cefoxitin	30 µg		24 – 30 mm
Trimeth.-Sulfam.	1,25/23,75 µg		26 – 32 mm

Conditions, Mueller-Hinton II Blood Agar, EUCAST:			
Incubation temperature:	35 °C	Incubation time:	16 h
Test strains		**Growth**	**Zone diameter**
Streptococcus pneumoniae **ATCC49619**		Good	
Oxacillin	1 µg		8 – 14 mm
Trimeth.-Sulfam.	1,25/23,75 µg		18 – 26 mm

Conditions, Mueller-Hinton II Chocolate Agar:			
Incubation temperature:	35 °C	Incubation time:	16 h
Test strains		**Growth**	**Zone diameter**
Haemophilus influenzae **ATCC49766**		Good	
Ciprofloxacin	5 µg		32 – 40 mm
Cefotaxime	5 µg		29 – 37 mm

References: Mueller and Hinton (1941) Proc. Soc. Exp. Biol. Med. 48: 330.
European Committee On Antimicrobial Susceptibilty Testing [www.eucast.org]
CLSI Performance Standards for Antimicrobial Disk Susceptibility Testing

In vitro diagnostic – for professional use only!