

MUELLER HINTON AGAR

IVD in Class A, EU Reg. 2017/746

 For in vitro diagnostic use **IVD**

Medium for antimicrobial susceptibility testing by the disc diffusion technique, according to CLSI and EUCAST.

DESCRIPTION

Mueller Hinton Agar is recommended for antimicrobial disc diffusion susceptibility testing of common, rapidly growing bacteria by the Bauer-Kirby method, as standardized by the Clinical and Laboratory Standards Institute (CLSI).

PRINCIPLE

Acid hydrolysate (digest) of casein and beef extract supply amino acids and other nitrogenous substances, minerals, vitamins, carbon and other nutrients to support the growth of microorganisms. Starch acts as a protective colloid against toxic substances that may be present in the medium. Hydrolysis of the starch during autoclaving provides a small amount of dextrose, which is a source of energy. Agar is the solidifying agent.

The Bauer-Kirby procedure is based on the diffusion through an agar gel of antimicrobial substances which are impregnated on paper discs. In contrast to earlier methods which used discs of high and low antimicrobial concentrations and which used the presence or absence of inhibition zones for their interpretation, this method employs discs with a single concentration of antimicrobial agent and zone diameters are correlated with minimal inhibitory concentrations (MIC).

In the test procedure, a standardized suspension of the organism is swabbed over the entire surface of the medium. Paper discs impregnated with specified amounts of antibiotic or other antimicrobial agents are then placed on the surface of the medium, the plate is incubated and zones of inhibition around each disc are measured. The determination as to whether the organism is susceptible, intermediate or resistant to an agent is made by comparing zone sizes obtained to those in the CLSI Document M100(M2).

Various factors have been identified as influencing disc diffusion susceptibility tests. These include the medium, excess surface moisture on the medium, agar depth, disc potency, inoculum concentration, pH and β -lactamase production by test organisms.

COMPOSITION	g/L
Beef Extract	2.0
Acid Hydrolysate of Casein	17.5
Starch	1.5
Agar	17.0

Final pH 7,3 ± 0,2 at 25°C

WARNING AND PRECAUTIONS

For in vitro diagnostic use.

Observe the precautions normally taken when handling laboratory reagents.

Dehydrated medium: HIGHLY HYGROSCOPIC. During the handling, wear dust protection mask. Avoid the eye contact. Do not use beyond the expiration date or if the product shows signs of deterioration, an altered color or has compacted.

Prepared Medium: The product does not contain hazardous substances in concentrations exceeding the limits set by current legislation and therefore is not classified as dangerous.

Safety Data Sheet is available on request for professional users.

All waste must be disposed of according to local directives.

STORAGE AND STABILITY

Dehydrated medium:	10-30°C
Prepared medium:	10-25°C

MUELLER HINTON AGAR is stable until the expiration date indicated on the label under the recommended storage conditions.

PREPARATION

Dehydrated medium: Suspend 38 g of the powder in 1 liter of distilled or deionized water. Mix thoroughly. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. Autoclave at 121°C for 15 minutes. Cool to 45-50°C. Aseptically dispense in Petri dishes on a level, horizontal surface to give a uniform depth of about 4 mm and cool to room temperature.

Prepared medium (bottles): Melt the content of the bottle in a water bath at 100°C until completely dissolved. Then screw the cap and check the homogeneity of the dissolved medium, if it is the case turning the bottle upside down. Cool at 45-50°C, mix well avoiding foam formation and aseptically distribute into Petri dishes.

Prepared medium (plates): ready to use.

PROCEDURE

1. Prepare a standardized suspension of the test organism using either the direct colony suspension or growth method.
2. Dip a sterile cotton swab into the adjusted suspension.
3. Inoculate the surface of the plate by streaking the swab over the entire agar surface.
4. Apply the antimicrobial discs onto the surface of the inoculated agar plate.
5. Incubate aerobically at 35 ± 2°C for 16-18 hours

It is also possible to use other protocols, provided that the inoculum and the seeding method are previously studied and standardized.

RESULTS

After incubation measure the diameter of the zone of complete inhibition including the diameter of the disc. Interpret zone sizes by referring to the current CLSI or EUCAST procedures and standards. Report the organism as susceptible, intermediate or resistant to the agents that have been tested.

QUALITY CONTROL

Dehydrated medium: Beige, free-flowing, homogeneous

Prepared medium: Light to medium amber, slightly opalescent

Typical response after incubation at 37°C for 24 hours, in aerobiosis

MICROORGANISM	GROWTH
Enterococcus faecalis ATCC® 33186	Luxuriant/Good
Escherichia coli ATCC® 25922	Luxuriant/Good
Escherichia coli ATCC® 35218	Luxuriant/Good
Pseudomonas aeruginosa ATCC® 27853	Luxuriant/Good
Staphylococcus aureus ATCC® 25923	Luxuriant/Good
Staphylococcus aureus ATCC® 43300	Luxuriant/Good

(*) For antibiotic susceptibility tests refer to NCCLS standards.

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PRESENTATION	Packaging	REF.
Dehydrated medium:		
MUELLER HINTON AGAR		
	100 g (2.6 L)	11110
	500 g (13.1 L)	10110
	5 Kg (131.5 L)	13110
Prepared medium:		
MUELLER HINTON AGAR		
	6 x 100 mL bottles	63322
	6 x 200 mL bottles	63222
	12 x 200 mL bottles	63522
	20 pcs (90 mm ready-to-use plates)	2154252/20
	10 pcs (150 mm ready-to-use plates)	2204253

SYMBOLS



Read the instructions



Biological hazard



CE Mark (product complies with the requirements of Regulation (EU) 746/2017)



Temperature limitation



Use by



For in vitro diagnostic use



Manufacturer