



## Mueller Hinton II Broth

Cation-adjusted Mueller Hinton broth for antimicrobial susceptibility testing of bacteria that grow aerobically.

### INTENDED PURPOSE

Liquid medium for quantitative antimicrobial susceptibility testing of rapidly growing aerobic organisms by broth dilution procedures, as standardized by the CLSI.

### DESCRIPTION

Mueller Hinton II Broth (MH II broth or cation-adjusted MHB) is the recommended medium for Minimum Inhibitory Concentration (MIC) testing of commonly isolated rapidly growing aerobic bacteria by broth dilution procedures.

This medium is formulated to have low levels of thymine and thymidine and is adjusted to the calcium and magnesium ions concentrations. It conforms to Clinical and Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendations.

### TYPICAL FORMULA\*

	(g/l)
Beef Extract	3.0
Acid Hydrolysate of Casein	17.5
Starch	1.5
Final pH 7.3 ± 0.1 at 25°C	

\*Adjusted and/or supplemented as required with appropriate salts to provide the following concentrations of ions and meet performance specifications according to ISO/TS 16782: 20-25 mg/l of calcium, 10-12.5 mg/l of magnesium, manganese and zinc below 8 mg/l and 3 mg/l, respectively.

### METHOD PRINCIPLE

Acid hydrolysate of casein and beef extract provide amino acids, nitrogen, minerals, vitamins, carbon and other nutrients which support the growth of microorganisms. These ingredients are selected for low thymine and thymidine content as determined by MIC values obtained by testing *Enterococcus faecalis* with trimethoprim-sulfamethoxazole (SXT). Starch acts as a protective colloid against toxic molecules which can be present in the medium. Hydrolysis of starch during autoclaving supplies a little amount of glucose, which is a source of energy. Specified levels of calcium and magnesium are based on studies performed with aminoglycosides and *Pseudomonas aeruginosa*.

Broth dilution methods, macrodilution and microdilution, are used to measure quantitatively the *in vitro* activity of an antimicrobial agent against a given bacterial isolate. To perform the test, a series of tubes/wells is prepared with a broth medium to which various concentrations of the antimicrobial agents are added. The tubes/wells are then inoculated with a standardized suspension of the test organism. After incubation at 35 ± 2°C, the tests are examined and the MIC is determined.

### MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as: inoculating loops, sterile cotton swabs, physiological solution, incubator, quality control organisms.

### TEST PROCEDURE

1. Prepare antimicrobial agents in serial two-fold dilutions in Mueller Hinton II Broth.
2. Prepare a standardized suspension of the test organism using either the direct colony suspension or growth method.
3. Add the suitable volume of the adjusted inoculum to each tube/well containing the antimicrobial agent in the dilution series, and mix.
4. Incubate the tubes or trays aerobically at 35 ± 2°C for 16-20 hours.

For more detailed information, consult appropriate guidance.

## INTERPRETING RESULTS

After incubation the presence of turbidity indicates growth of the organism. The lowest concentration of antimicrobial agent showing no growth is the MIC of that organism for that agent.

Interpret the MIC by referring to the current CLSI document M100 and report the organism as susceptible, intermediate or resistant to the agents that have been tested.

## STORAGE

Store at 10-25°C away from light. Do not use the product beyond its expiry date on the label or if product shows any evidence of contamination or any sign of deterioration.

## SHELF LIFE

2 years

## QUALITY CONTROL

**Appearance of medium:** Pale to light yellow, clear to slightly hazy.

**Expected Cultural Response:**

Control strains		Inoculum	Incubation	Criteria
<i>Enterococcus faecalis</i>	WDCM 00087 (ATCC® 29212; NCTC 12697)	10-100 CFU/ml	16 - 20 h / 35 ± 2°C	Good growth
<i>Escherichia coli</i>	WDCM 00013 (ATCC® 25922; NCTC 12241)			
<i>Pseudomonas aeruginosa</i>	WDCM 00025 (ATCC® 27853; NCTC 12903)			
<i>Staphylococcus aureus</i>	WDCM 00131 (ATCC® 29213; NCTC 12973)			

## PERFORMANCE CHARACTERISTICS

Performance testing of Mueller Hinton II Broth was carried out using the QC strains listed above. The results obtained met the established criteria.

## LIMITATIONS

Invalid results can be caused by poor sample quality, improper sample collection, improper transportation, improper laboratory processing, or a limitation of the testing technology. The operator should understand the principles of the procedures, including its performance limitations, in advance of operation to avoid potential mistakes.

Due to nutritional variation, some strains may result in poor growth or fail to grow in this medium. For example, microorganisms requiring thymine and thymidine may not grow. Also, fastidious organisms such as species of *Streptococcus*, *Haemophilus*, and *Neisseria* will not grow or will grow poorly in MH II Broth.

The efficacy of this medium has not been established for all microorganisms that might be isolated from sample. If growth is inadequate (i.e. turbidity that cannot be seen by the naked eye) the MIC values may not be valid. Always include a growth control tube or well that contains the inoculated medium but no antimicrobial agent. If no growth is seen, repeat testing or use an alternative procedure.

*In vitro* susceptibility of an organism to a specific antimicrobial agent does not mean that it will be effective as a therapeutic agent *in vivo*.

MH II broth is not adequate for susceptibility testing of fastidious organisms. For MIC tests of fastidious organisms such as *Haemophilus influenzae*, *Neisseria gonorrhoeae* and *Streptococcus pneumoniae*, use proper media according to the reference method.

## WARNING AND PRECAUTIONS

**For professional use only.** Operators must be trained and have certain experience. Please read the instructions carefully before using this product. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this document.

Consult the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.

## DISPOSAL OF WASTE

Disposal of waste must be carried out according to national and local regulations in force.

## BIBLIOGRAPHY

See the references at the end of this document.

## TABLE OF SYMBOLS

See the table of symbols at the end of this document.

## ORDER INFORMATION

Product	Format	Packaging	Ref.
Mueller Hinton II Broth	16x100mm Plastic Tube	100 x 3 ml	26510P
	20x100mm Tube	20 x 3 ml	27510

## Revision History

Revision	Release Date	Change Summary
0	2025-11-14	Document creation

This IFU document and the SDS are available from the online Support Center:

[liofilchem.com/ifu-sds](http://liofilchem.com/ifu-sds)

## References

1. EN ISO 11133:2014+Amd1:2018+Amd2:2020. Microbiology of food, animal feed and water – Preparation, production, storage and performance testing of culture media.
2. ISO/TS 16782:2016. Clinical laboratory testing — Criteria for acceptable lots of dehydrated Mueller-Hinton agar and broth for antimicrobial susceptibility testing.
3. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 35th ed. CLSI Supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2025.
4. CLSI. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically. 12th ed. CLSI standard M07. Clinical and Laboratory Standards Institute; 2024.
5. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 15.0, 2025. <http://www.eucast.org>.
6. The European Committee on Antimicrobial Susceptibility Testing. Routine and extended internal quality control for MIC determination and disk diffusion as recommended by EUCAST. Version 15.0, 2025. <http://www.eucast.org>

## Table of Symbols

	Batch code
	Catalogue number
	Manufacturer
	Use by
	Fragile, handle with care
	Temperature limitation
	Contains sufficient for <n> tests
	Consult instructions for use
	Do not reuse
	Keep away from sunlight



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