

# Mueller Hinton Broth (Not Cation-Adjusted)

## Intended Use

Mueller Hinton Broth is a general purpose medium that may be used in the cultivation of a wide variety of fastidious and non-fastidious microorganisms. This medium is not supplemented with calcium or magnesium ions.

## Summary and Explanation

The Mueller Hinton formulation was originally developed as a simple, transparent agar medium for the cultivation of pathogenic *Neisseria*.<sup>1</sup> Other media were developed that replaced the use of Mueller Hinton Agar for the cultivation of pathogenic *Neisseria*, but it became widely used in the determination of sulfonamide resistance of gonococci and other organisms. It is now used as a test medium for antimicrobial susceptibility testing.<sup>2</sup>

Mueller Hinton Broth, not cation-adjusted, has a formula similar to that of the solid medium, but without agar, for use when the fluid medium is preferred. While it may be used for the general cultivation of bacteria, for consistency, cation-adjusted Mueller Hinton Broth is now recommended for dilution antimicrobial susceptibility testing of all species of most commonly encountered aerobic and facultatively anaerobic

bacteria.<sup>2,3</sup> BBL™ Mueller Hinton II Broth is cation-adjusted to the calcium and magnesium ion concentrations recommended in the CLSI standard M7.<sup>2</sup>

Difco™ Mueller Hinton Broth, not cation-adjusted, is formulated to have a low thymine and thymidine content. It may be used for broth dilution antimicrobial susceptibility testing as long as the calcium and magnesium ion concentrations are adjusted according to CLSI standard M7.<sup>2</sup>

BBL™ Mueller Hinton Broth, not cation-adjusted, has not been formulated to have a low thymine and thymidine content. It may be used for the general cultivation of bacteria.

## Principles of the Procedure

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.

## User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both Difco™ and BBL™ brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

### Identity Specifications

#### Difco™ Mueller Hinton Broth

Dehydrated Appearance:	Light beige, free-flowing, homogeneous with a few dark specks.
Solution:	2.1% solution, soluble in purified water upon boiling. Solution is very light amber, clear, may have a slight precipitate.
Prepared Appearance:	Very light amber, clear, may have a slight precipitate.
Reaction of 2.1% Solution at 25°C:	pH 7.3 ± 0.1
Calcium:	2.9-5.9 mg/L
Magnesium:	3.2-5.2 mg/L

### Cultural Response

#### Difco™ Mueller Hinton Broth

Prepare the medium per label directions, supplementing with calcium and magnesium ions according to CLSI standard M7.<sup>2</sup> Prepare broth micro-dilution trays, inoculate (with the organisms listed below) and incubate as recommended by CLSI.<sup>2</sup> Compare the MIC (lowest concentration of antimicrobial that inhibits growth of the test bacterium) of the antimicrobials tested to the CLSI standard.<sup>2</sup>

ORGANISM	ATCC™
<i>Enterococcus faecalis</i>	29212
<i>Escherichia coli</i>	25922
<i>Pseudomonas aeruginosa</i>	27853
<i>Staphylococcus aureus</i>	29213

### Identity Specifications

#### BBL™ Mueller Hinton Broth

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	2.2% solution, soluble in purified water upon boiling. Solution is pale to light, tan to yellow, clear to slightly hazy.
Prepared Appearance:	Pale to light, tan to yellow, clear to slightly hazy.
Reaction of 2.2% Solution at 25°C:	pH 7.3 ± 0.1

### Cultural Response

#### BBL™ Mueller Hinton Broth

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours (up to 72 hours, if necessary).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
<i>Enterococcus faecalis</i>	29212	≤10 <sup>3</sup>	Good
<i>Enterococcus faecalis</i>	33186	≤10 <sup>3</sup>	Good
<i>Escherichia coli</i>	25922	≤10 <sup>3</sup>	Good
<i>Pseudomonas aeruginosa</i>	27853	≤10 <sup>3</sup>	Good
<i>Staphylococcus aureus</i>	29213	≤10 <sup>3</sup>	Good

## Formulae

### Difco™ Mueller Hinton Broth

Approximate Formula* Per Liter	
Beef Extract Powder .....	2.0 g
Acid Digest of Casein .....	17.5 g
Starch .....	1.5 g

### BBL™ Mueller Hinton Broth

Approximate Formula* Per Liter	
Beef Extract.....	3.0 g
Acid Hydrolysate of Casein.....	17.5 g
Starch .....	1.5 g

\*Adjusted and/or supplemented as required to meet performance criteria.

## Directions for Preparation from Dehydrated Product

1. Suspend the powder in 1 L of purified water:  
**Difco™ Mueller Hinton Broth – 21 g;**  
**BBL™ Mueller Hinton Broth – 22 g.**  
Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 116-121°C for 10-15 minutes (consult product label). DO NOT OVERHEAT.
4. Check prepared medium to ensure the final pH is 7.3 ± 0.1 at 25°C.
5. Test samples of the finished product for performance using stable, typical control cultures.

## Procedure

For a complete discussion on broth dilution antimicrobial susceptibility testing, refer to the appropriate procedures outlined in the references.<sup>2-5</sup>

Organisms to be subcultured must first be isolated in pure culture on an appropriate solid medium. Transfer growth from the isolation medium to Mueller Hinton Broth using standard bacteriologic techniques.<sup>3,4</sup>

For enrichment purposes, inoculate the specimen onto primary media and then into the broth, according to recommended procedures.

Incubate the tubes at 35°C under conditions appropriate for the organism being cultured.

## Expected Results

For broth dilution antimicrobial susceptibility testing, refer to appropriate references for results.<sup>2,5</sup>

Growth in broth media is indicated by the presence of turbidity compared with an uninoculated control.

## References

1. Mueller and Hinton. 1941. Proc. Soc. Exp. Biol. Med. 48:330.
2. Clinical and Laboratory Standards Institute. 2006. Approved Standard: M7-A7. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 7th ed. CLSI, Wayne, Pa.
3. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
4. Forbes, Sahm and Weissfeld. 2007. Bailey & Scott's diagnostic microbiology, 12th ed. Mosby, Inc., St. Louis, Mo.
5. Isenberg and Garcia (ed.). 2004 (update, 2007). Clinical microbiology procedures handbook, 2nd ed. American Society for Microbiology, Washington, D.C.

## Availability

### Difco™ Mueller Hinton Broth (Not cation-adjusted)

#### CLSI

Cat. No.	275730	Dehydrated – 500 g
	275710	Dehydrated – 2 kg

### BBL™ Mueller Hinton Broth (Not cation-adjusted)

Cat. No.	211443	Dehydrated – 500 g
	296195	Prepared Tubes, 2 mL (K Tubes) – Pkg. of 10
	296164	Prepared Tubes, 2 mL (K Tubes) – Ctn. of 100
	297220	Prepared Tubes, 5 mL (C Tubes) – Pkg. of 10
	295834	Prepared Tubes, 5 mL (C Tubes) – Ctn. of 100

#### Europe

Cat. No.	257092	Prepared Bottles, 900 mL – Pkg. of 4
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