Mueller Hinton Agar Plate (Gamma-Irradiated)

Intended Use
Mueller Hinton Agar Plate is used for antimicrobial disc diffusion susceptibility testing of common, rapidly growing bacteria by Kirby-Bauer method.

Summary
Mueller Hinton Agar was originally developed for the cultivation of *Neisseria.* These organisms are now isolated on selective media. Since clinical laboratories were using a wide variety of procedures for determining the susceptibility of bacteria to antibiotic and chemotherapeutic agents, Bauer, Kirby and others developed a standardized procedure in which Mueller Hinton Agar was selected as the test medium. Subsequently, international collaborative study confirmed the value of Mueller Hinton Agar for this purpose due to its relatively good reproducibility, the simplicity of its formula, and the wealth of experimental data that had been accumulated using this medium. Mueller Hinton Agar complies with the requirements of World Health Organization and is specified in the FDA's Bacteriological Analytical Manual for food testing.
The Kirby-Bauer antimicrobial disk diffusion procedure is used with Mueller Hinton Agar plates. It is based on the use of an antimicrobial impregnated filter paper disk. The impregnated disk is placed on an agar surface, resulting in diffusion of the antimicrobial into the surrounding medium. Effectiveness of the antimicrobial can be shown by measuring the zone of inhibition for a pure culture of an organism. Zone diameters established for each antimicrobial determining resistant, intermediate, and sensitive results for pathogenic microorganisms are listed in the Clinical and Laboratory Standards Institute (CLSI). For additional details refer to The National Committee for Clinical Laboratory Standards (NCCLS) which contains the performance standard for the Bauer-Kirby procedure. This procedure is recommended for testing rapidly growing aerobic or facultative anaerobic bacterial pathogens, such as Staphylococci, members of the *Enterobacteriaceae*, aerobic gram-negative rods, e.g. *Pseudomonas* species and *Acinetobacter* species, Enterococci and *Vibrio cholerae*. The procedure is modified for testing fastidious species; i.e. *H. influenza, N. gonorrhoeae, S. pneumoniae* and other Streptococci. The NCCLS Document M2, Performance for Antimicrobial Disc Susceptibility Tests, recommends Mueller Hinton Agar supplemented with 5% defibrinated sheep blood for fastidious organisms.

Principle
Casein acid hydrolysate and beef extract powder 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 starch during autoclaving provides a small amount of dextrose, which is a source of energy.

Formula*

Ingredients	**g/L**
Casein Acid Hydrolysate	17.5 g
Beef Extract Powder	2.0 g
Starch	1.5 g
Agar	17.0

*Adjusted to suit performance parameters.

Additional Material Required
Bacteriological Incubator.

Instructions for use
1. Open the sterile pack and remove Mueller Hinton Agar Plate aseptically.
2. Inoculate/streak the plate, place required antibiotic disc and incubate in inverted position as per standard procedure.

Reading and interpretation
1. After incubation, observe the microbial growth and count the colonies.
2. Interpretation is assured by user.

Quality Control

Appearance: Gel with smooth and even surface, without any cracks, bubbles and drying or shrinking of media.
Colour of Medium: Light amber coloured, very slightly opalescent gel in petriplates.
Quantity of Medium: 26 ± 2 g in 90 mm petriplate.
pH at 25°C ± 2°C: 7.3 ± 0.1
Gamma Irradiation : The above said product was Gamma Irradiated between 12 KGy - 21 KGy.
Growth Promotion Test: Growth promotion is carried out in accordance with the harmonized method of USP/EP/JP and growth is observed after an incubation at 30-35°C for 18-24 hours.
Growth Promoting Properties: The test results observed are within the specified temperature and shortest period of time specified in the test, inoculating ≤ 100 cfu of appropriate microorganism.

Organism (ATCC)	**Growth**
Staphylococcus aureus subsp. *aureus* (25923)	Good
Escherichia coli (25922)	Good
Enterococcus faecalis (29212)	Good
Pseudomonas aeruginosa Strain Boston 41501 (27853)	Good

Precautions/Limitations

1. Un supplemented Mueller Hinton Agar, although adequate for susceptibility testing of rapidly growing aerobic pathogens, is not adequate for more fastidious organisms such as *S. pneumoniae.*
2. Numerous factors can affect the result: inoculum size, rate of growth, media formulation, pH, length of incubation, disc content, drug diffusion rates, and measurement of endpoints. Hence, strict adherence to protocol is required to ensure reliable results.
3. Mueller Hinton Agar deeper than 4 mm may cause false resistant results, and agar less than 4 mm deep may be associated with a false-susceptibility report.
4. pH outside the range of 7.3 ± 0.2 may adversely affect susceptibility test results. If the pH is too low, aminoglycosides and macrolides will appear to lose potency; others may appear to have excessive activity. The opposite effects are possible if the pH is too high.
5. The following technical and human errors may occur which compromise on reliability and accuracy and must be avoided: -

· Improper disc storage.
· Inoculum not properly adjusted (too light or too heavy).
· Incubation temperature deviating from 30-35° C.
· Use of an increased CO_2 atmosphere. Reading plates before or after the full 16-18 hours of incubation.
· Transcribing errors.
· Reading error while measuring zone diameter.
· Deterioration of the McFarland Turbidity Standard.

Storage and Shelf Life

1. Store between 15°C-25°C to avoid water condensation. Condensation can be prevented by avoiding quick temperature shifts and mechanical stress.
2. Under optimal conditions, the medium has a shelf life of 6 months. Use before expiry mentioned on the label.

Reference

1. Mueller and Hinton, 1941, Proc. Soc. Exp. Bio. And Med; 48:330.
2. Bauer et al.,1966, Am. J. Clin. Patho., 45:493.
3. US Food and Drug Adm; 1998, Bacteriological Analytical Manual, 8th Ed; Rev. A, AOAC, International, Gaithersburg, Md.
4. National Committee for Clinical Laboratory Standards. 2000. Approved Standard: M2-A7. Performance Standards For
5. Antimicrobial Disk Susceptibility Tests, 7th edition. NCCLS, Wayne, P.A.
6. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Yolken R. H., (Ed.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.
7. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat No.	Product	Pack Size
205131790100	Mueller Hinton Agar Plate	100 Plates
205131790020	Mueller Hinton Agar Plate	20 Plates