MUELLER HINTON AGAR

- For in vitro use only - Catalogue No. PM90 (K)

Our Mueller Hinton Agar is the recommended medium for the antimicrobial disk diffusion testing of common, aerobic, rapidly growing bacteria.

Mueller Hinton was originally devised as a medium for culturing *Neisseria* species. Later on in the 1960's, Kirby, Bauer and other researchers attempted to standardize the procedure used in susceptibility testing of bacteria and selected Mueller Hinton Agar as the ideal medium. The Kirby-Bauer procedure is based on the use of paper disks impregnated with antimicrobial agents. The antimicrobial agents diffuse into the agar medium resulting in various zones of inhibition depending on the organism being tested. Today, the Clinical and Laboratory Standards Institute (CLSI) puts forth the standardized guidelines for the use and interpretation of Mueller Hinton Agar. The CLSI document M2, *Performance Standards for Antimicrobial Disk Susceptibility Tests*, provides procedures for the testing of rapidly growing aerobic and facultatively anaerobic bacteria, which include members of the *Enterobacteriaceae, Staphylococcus* spp., *Enterococcus* spp., *Pseudomonas* spp., *Acinetobacter* spp., and *Vibrio cholerae*. A modified medium and testing procedure is also provided for more fastidious species such as *Haemophilus influenzae, Neisseria gonorrhoeae,* and *Streptococcus pneumoniae*.

Formulation per Litre of Medium

Beef Extract	2.0 g
Casein Hydrolysate	17.5 g
Starch	1.5 g
Agar	17.0 g

pH 7.3 ± 0.1

Recommended Procedure

1. Mueller Hinton Agar and the required antibiotic disks should be removed from the refrigerator or freezer 1 to 2 hours before use so that they may equilibrate to room temperature. Remove Mueller Hinton Agar from packaging to allow excess moisture to dissipate from the surface during this warm-up period.
2. From a pure culture plate, select at 3 to 5 well-isolated colonies of the sample morphology. Touch the tops of each colony and transfer to a tube containing 4 to 5 mL of a suitable broth medium.
3. Incubate broth at 35˚C until it achieves or exceeds the turbidity of a 0.5 McFarland Standard (equivalent to $1.5x10^8$ CFU/mL). If required, adjust the turbidity of the broth to match that of the 0.5 McFarland standard using sterile saline or broth. This suspension must be used within 15 minutes of its adjustment to ensure accuracy.
4. Dip a sterile swab into the suspension and roll the swab firmly against the side of the tube several times to remove excess inoculum form the swab.
5. Use the swab to streak the entire surface of the Mueller Hinton Agar plate. Repeat the streaking procedure two more times, rotating the plate approximately 60˚ each time to ensure even distribution of the inoculum. The inoculated plate may be left for 5 minutes to allow the surface inoculum to be adsorbed.
6. Using aseptic technique, the selected antimicrobial disks are evenly distributed on the agar surface individually or with a dispensing apparatus. Press down gently on each disk to ensure that complete contact with the agar surface is obtained.

7. Invert plates and incubate at 35˚C for 16 to 18 hours.
8. After the incubation period, examine and interpret plates.

Interpretation of Results

After the incubation period, a confluent lawn of growth should be obtained; if individual colonies are observed instead than the inoculum was too light and the test must be repeated. A zone of inhibition should be observed around each antimicrobial disk. Using a ruler or calipers measure the zones on the back of the plate to the nearest whole millimeter. When measuring the zones, the plate should be held over a black, non-reflecting background, and illuminated from above. The zone margin or endpoint should be taken as the area showing no obvious visible growth that can be detected with the unaided eye. Disregard faint growth of tiny colonies that can be detected with difficulty near the edge of the obvious zone of inhibition with the exception of staphylococci and enterococci. In these cases, direct, transmitted light should be used to detect methicillin and vancomycin resistant strains indicated by light growth within the apparent zones of inhibition.

To accurately determine susceptibility results use updated CLSI tables of antimicrobial disks and interpretative standards. The complete standard and informational supplements can be ordered directly from the Clinical and Laboratory Standards Institute; visit clsi.org for more information.

- *With Proteus species, if the zone of inhibition is distinct enough to measure, disregard any swarming inside the zone.*

- *Numerous factors can affect results and zone sizes: inoculum size; rate of growth; pH; length of incubation and incubation environment; disk content and drug diffusion rate; and measurement of endpoints. Therefore strict adherence to CLSI testing protocols are essential for obtaining accurate and reproducible results.*

- *A maximum of 5 antimicrobial disks are prescribed for the regular 100-mm size plates, while no more than 12 disks should be placed on the Kirby 150-mm plates.*

Quality Control

Internal monitoring and testing of each lot of Mueller Hinton Agar is required as outlined by the CLSI. Each time a new lot of agar or a new lot of antimicrobial disks is introduced, it must be tested with the appropriate quality control strains. For more information please refer to The CLSI document M2, *Performance Standards for Antimicrobial Disk Susceptibility Tests.*

Storage and Shelf Life

Our Mueller Hinton Agar should be stored at 4°C to 8°C and protected from light. The medium side should be uppermost to prevent excessive accumulation of moisture on the agar surface. Under these conditions the medium has a shelf life of 10 weeks from the date of manufacture.

Ordering Information

Cat#	Description	Format
PM90	Mueller Hinton Agar [Standard 15x100-mm plate]	10/pkg
PM90K	Mueller Hinton Agar [Kirby 15x150-mm plate]	5/pkg

References

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9. Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH. Manual of clinical microbiology. 7th ed. Washington D.C.: ASM, 1999.
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