MUELLER HINTON AGAR

with 2% Glucose and Methylene Blue

- For in vitro use only - Catalogue No. PM85

Our Mueller Hinton Agar with glucose and methylene blue is the recommended medium for the antimicrobial disk diffusion testing of yeasts, and more specifically for *Candida* species.

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 addition of glucose and methylene blue indicator is the recommended media for the susceptibility testing of *Candida* species according to the CLSI M44-A2 document. The presence of glucose serves as an energy source for fungal cultures while the methylene blue enhances zone definition.

Formulation per Litre of Medium

Beef Extract	2.0 g
Casein Hydrolysate	17.5 g
Starch	1.5 g
Glucose	20.0 g
Methylene Blue	0.0005 g
Agar	17.0 g

pH 7.3 ± 0.1

Recommended Procedure

1. Mueller Hinton Agar plates 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 plates 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 5 mL of sterile saline.
3. Adjust the solution until it achieves the turbidity of a 0.5 McFarland Standard (equivalent to $1.0x10^6$ to $5.0x10^6$ CFU/mL). If required, adjust the turbidity of the broth to match that of the 0.5 McFarland standard using sterile saline or additional growth. Ideally, use suspension 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 20 to 24 hours.
8. After the incubation period, examine and measure visible zones. Re-incubate plates an additional 24 hours if insufficient growth is observed.

Interpretation of Results

After the incubation period, satisfactorily streaked plates will show a semi-confluent lawn of growth along with uniformly circular zones of inhibition around each disk; 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.

To accurately determine susceptibility results, use updated CLSI tables of antimicrobial disks and interpretative standards.

- *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 plate.*

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 M44, *Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts.*

Storage and Shelf Life

Our Mueller Hinton Agar with 2% Glucose and Methylene Blue should be stored at 4 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 8 weeks from the date of manufacture.

Ordering Information

Cat#	Description	Format
PM85	Mueller Hinton Agar w/ Glucose & Methylene Blue [Standard 15x100-mm plate]	10/pkg

References

1. Mueller JH, Hinton J. A protein-free medium for primary isolation of Gonococcus and Meningococcus. Proc Soc Exp Biol Med 1941; 48:330-3.
2. Bauer AW, Sherris JC. The determination of sulfonamide susceptibility of bacteria. Chemotherapia 1964; 9:1-19.
3. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized disc method. Am J Clin Pathol 1966; 493-6.
4. MacFaddin JF. Media for isolation, cultivation, identification, maintenance of bacteria, Vol I. Baltimore: Williams & Wilkins, 1985.
5. NCCLS. Protocols for evaluating dehydrated Mueller-Hinton agar; approved standard. M6-A. Wayne, PA: NCCLS, 1996.
6. Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH. Manual of clinical microbiology. 7th ed. Washington D.C.: ASM, 1999.
7. CLSI. Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts; Approved Guideline- 2nd ed. M44-A2. Wayne, PA: CLSI, 2012.

Original: November 2005
Revised / Reviewed: April 2025