

Mueller Hinton Agar With 2% Glucose and Methylene Blue

Intended Use

Mueller Hinton Agar with 2% Glucose and Methylene Blue is recommended for performing Antifungal Disc Diffusion Susceptibility testing of yeast.

Summary

The Mueller Hinton formulation was originally developed as a simple, transparent agar medium for the cultivation of pathogenic species. Mueller Hinton Agar, modified (as per CLSI for antifungal) is recommended for the diffusion of antifungal agents impregnated on paper disc through an agar gel as described in CLSI Approved Standard. Kirby-Bauer *et al.*, recommended Mueller Hinton Agar for performing antibiotic susceptibility tests using a single disc of high concentration. WHO Committee on Standardization of Susceptibility Testing has accepted Mueller Hinton Agar for determining the susceptibility of microorganisms because of its reproducibility. When supplemented with glucose to a final concentration of 2%, it provides for suitable fungal growth. The addition of methylene blue to a final concentration of 5 µg/mL enhances zone edge definition.

Principle

Beef infusion and casein acid hydrolysate provide nitrogenous compounds, carbon, sulphur and other essential nutrients. Starch acts as a protective colloid against toxic substances present in the medium. Starch hydrolysis yields dextrose, which serves as a source of energy. Glucose serves as an energy source for fungal cultures while Methylene blue enhances zone edge definition.

Formula*

Ingredients	g/L
Beef infusion from 300.0	2.0
Casein Acid Hydrolysate	17.5
Starch	1.5
Glucose	20.0
Methylene Blue	0.0005
Agar	17.0
Final pH (at 25°C)	7.3 ± 0.1

*Adjusted to suit performance parameters.

Storage and Stability

Store dehydrated medium below 30°C in tightly closed container and the prepared medium at 2°C-8°C. Avoid freezing and overheating. Use before expiry date on the label. Once opened keep powdered medium closed to avoid hydration.

Specimen Collection and Handling

Ensure that all samples are properly labelled. Follow appropriate techniques for handling samples as per established guidelines. Some samples may require special handling, such as immediate refrigeration or protection from light, follow the standard procedure. The samples must be stored and tested within the permissible time duration. After use, contaminated materials must be sterilized by autoclaving before discarding.

Directions

1. Suspend 58.00 g of the powder in 1000 mL purified / distilled water.
2. Heat to boiling to dissolve the powder completely.
3. Sterilize by autoclaving at 121°C (15 psi) for 15 minutes as per validated cycle.
4. Mix well and pour into sterile petridishes. Or dispense as desired.

Quality Control

Dehydrated Appearance: Light yellow to yellow coloured, may have slight blue tinge homogeneous, free flowing powder.

Prepared Appearance: Amber coloured, clear to very slightly opalescent gel forms in petridishes.

Cultural Response: A Good growth of test organisms is observed on Mueller Hinton Agar, 2% Glucose with Methylene Blue (as per CLSI for antifungal) in 24-48 hours at 33°C- 37°C along with the respective antibiotics concentrations.

Organism (ATCC)	Growth
<i>Candida albicans</i> (90028)	Good
<i>Candida parapsilosis</i> (22019)	Good
<i>Candida tropicalis</i> (750)	Good
<i>Candida krusei</i> (6258)	Good
<i>Candida albicans</i> 3147 (10231)	Good
<i>Saccharomyces cerevisiae</i> NRRL Y-567 (9763)	Good

Performance and Evaluation

Performance of the product is dependent on following parameters as per product label claim:

1. Directions
2. Storage
3. Expiry

Warranty

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

Reference

1. Mueller J. H. and Hinton J., 1941, Proc. Soc. Exp. Biol. Med., 48:330.
2. Method for Antifungal Disk Diffusion Susceptibility Testing of yeasts; Approved Guideline Second Edition M44-A2 Vol.24 No.17.
3. Present Status and Future Work, WHO Sponsored collaborative study, Chicago, Oct. 1967.
4. Bauer A. W., Kirby W. M., Sherris J. L. and Turck M., 1966, Am. J. Clin. Pathol., 45:493.
5. Data on file: Microexpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat No.	Product description	Pack Size
201130670500	Dehydrated Culture Media	500 g

Disclaimer

Information provided is based on our inhouse technical data on file, it is recommended that user should validate at his end for suitable use of the product.
