



MUELLER HINTON AGAR with 4 µg/mL CEFOXITIN

- For in vitro use only -

Catalogue No. PM88

Our Mueller Hinton Agar with 4 µg/mL of Cefoxitin is used for the detection of methicillin-resistant *Staphylococcus aureus* (MRSA).

Mueller Hinton Agar contains casein peptone and beef extract, two excellent nutritional sources that supply bacteria with all the essential elements needed for uniform growth. The addition of starch helps neutralize toxic substances in the medium as well as toxic metabolites produced during bacterial growth.

Our Mueller Hinton Agar is supplemented with 4 µg/mL of cefoxitin. Cefoxitin is a semisynthetic cephalosporin that has demonstrated its usefulness in the screening of MRSA. The NCCLS (CLSI) has recommended the use of a cefoxitin disc test as a confirmatory test for oxacillin resistance, and this test has also proven to be an accurate predictor of the *mecA* gene. The incorporation of cefoxitin into a medium (ie. mannitol salt agar) was first reported by Smyth and Kahlmeter in 2005; since then many other MRSA screening mediums have adopted cefoxitin as the drug of choice for the screening of MRSA due to its stability, high sensitivity and high specificity.

Growth on our Mueller Hinton Agar with cefoxitin indicates resistance and the results obtained correlate almost 100% with other tests that detect the presence of the gene responsible for resistance, *mecA*.

Formula per Litre of Medium

Beef Extract2.0 g
Casein Peptone.....17.5 g
Starch.....1.5 g

Agar.....17.0 g
Cefoxitin.....4.0 mg

pH 7.3 ± 0.2

Recommended Procedure

1. Allow medium to reach room temperature.
2. If coagulase-positive staphylococci are recovered from the primary plate, an inoculum equivalent to a 0.5 McFarland turbidity standard should be prepared in tryptic soy broth.
3. Using a sterile swab, dip it into the prepared suspension and either spot inoculate or streak the surface of the medium.
4. Incubate aerobically at 35°C.
5. Examine after 24 hours of incubation.

Interpretation of Results

Any growth on the plate is considered a presumptive positive for MRSA. Typically, MRSA colonies appear as small pinpoint colonies on the agar surface. A MIC determination via E-test™ or broth/agar dilution method can also be performed on all potentially resistant isolates.

Additional biochemical and serological tests should be performed on isolated colonies from pure culture in order to complete identification.

- A full and precise 24 hour incubation period is critical for accurate results

Quality Control

After checking for correct pH, color, depth, and sterility, the following organisms are used to determine the growth performance of the completed medium.

Organism	Expected Result
<i>Staphylococcus aureus</i> ATCC 43300	Growth
<i>Staphylococcus aureus</i> ATCC 25923	Complete Inhibition
<i>Staphylococcus aureus</i> ATCC 29213	Complete Inhibition

Storage and Shelf Life

Our Mueller Hinton Agar with 4 µg/mL of Cefoxitin stored away from direct light at 4 to 8°C with the medium side uppermost to prevent excessive accumulation of moisture on the agar surface. Under these conditions this medium has a shelf life of 6 weeks from the date of manufacture.

References

1. Mueller JH, Hinton J. A protein-free medium for primary isolation of gonococcus and meningococcus. Proc Soc Exp Biol Med 1941; 330-3.
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3. NCCLS. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 6th ed. Approved standard M7-A6. Wayne, PA: NCCLS, 2003.
4. NCCLS. Performance standards for antimicrobial disk susceptibility tests, 8th ed. Approved standard M2-A8. Wayne, PA: NCCLA, 2003.
5. NCCLS. Performance standards for antimicrobial susceptibility testing. Fourteenth informational supplement M100-S14. Wayne, PA: NCCLS, 2004.
6. Smyth RW, Kahlmeter G. Mannitol salt agar-cefoxitin combination as a screening medium for methicillin-resistant *Staphylococcus aureus*. J Clin Microbiol 2005; 43:3797-99.
7. Swenson JM, Tenover FC et al. Results of disk diffusion testing with cefoxitin correlate with the presence of the *mecA* in *Staphylococcus* spp. J Clin Microbiol 2005; 43:3819-23.
8. Fernandes CJ, Fernandes LA, Collignon P. Cefoxitin resistance as a surrogate marker for the detection of methicillin-resistant *Staphylococcus aureus*. J Antimicrob Chem 2005; 55:506-10.
9. Stoakes L et al. Prospective comparison of a new chromogenic medium, MRSASelect, to CHROMagar MRSA and mannitol-salt medium with oxacillin or cefoxitin for detection of methicillin-resistant *Staphylococcus aureus*. J of Clin Microbiol 2006; 44:637-9.

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