



Product Sheet

Methanohalophilus mahii (ATCC® 35705™)

Please read this **FIRST**



Intended Use

This product is intended for research use only. It is not intended for any animal or human therapeutic or diagnostic use.

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: *Methanohalophilus mahii* (ATCC® 35705™)

Description

Designation: SLP

Deposited Name: *Methanohalophilus mahii* Paterek and Smith

Propagation

Medium

ATCC® Medium 2485: MH-OCM Medium

Growth Conditions

Temperature: 37.0°C

Atmosphere: Anaerobic; 80% H₂ - 20% CO₂

Propagation Procedure

1. Sterilize the top of the Balch tube by spraying it with 70% ethanol and then flaming the top.
2. If needed exchange the gas in the test tube for 80% H₂-20% CO₂
3. If the medium is pink (see discussion about resazurin) add 0.1 ml of reducing agent (3% cysteine, stock solution) per each 10 ml of medium. Let the medium sit at room temperature for 30 minutes, until the resazurin becomes colorless, before inoculating.
4. Allow the frozen vial to thaw under anaerobic conditions. Once thawed, take a gassed 1.0 ml syringe tipped with 22-gauge needle and withdraw the entire contents of the thawed vial and immediately transfer it to a #2485 broth tube.
5. Plate 0.1 ml on a non-selective medium to check for aerobic and anaerobic contamination.
6. Incubate tubes and one plate under an anaerobic atmosphere at 37°C. Incubate non-selective plate aerobically at 37°C to check for purity.
7. In 72 to 96 hours, growth should be evident by light turbidity in the broth. No growth should occur on the non-selective plate incubated aerobically or anaerobically. No growth should occur on blood agar plates.

ANAEROBIC CONDITIONS:

- a. Balch tubes (available from Bellco Glass, Vineland, NJ) are specially designed for anaerobic work and use an aluminum crimp cap to hold a rubber stopper in place. Needles can easily be inserted through the stopper, and the tubes can be pressurized to 2 atm. Alternatively, serum vials may be used, or screw cap tubes with butyl rubber stoppers, in the latter case the stopper may be removed and the tube placed under a cannula system that dispenses sterile, oxygen free gas for addition of reducing agents or inoculation.
- b. Resazurin is a commonly used redox indicator that is pink when the redox potential is above 50 mv, and colorless when the redox potential is below 110 mv. i.e. highly reducing. Most strict anaerobes require this low redox potential for optimum growth.
- c. To obtain a fully reduced medium, it is necessary that the medium be anoxic and that a reducing agent be added. Common reducing agents are sodium sulfide, cysteine, dithiothreitol, and titanium citrate.

Notes

Within 96 hours of incubation, there should be turbidity in the broth. Cell morphology is irregular coccoid cells with no motility. This organism can utilize 20 mM of trimethylamine as a secondary substrate, which would promote growth.

Additional information on this culture is available on the ATCC® web site at www.atcc.org.

References

References and other information relating to this product are available online at www.atcc.org.

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the current publication of the *Biosafety in Microbiological and Biomedical Laboratories* from the U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes for Health.

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Biosafety Level
1

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