



Product Sheet

Methanosarcina mazei (ATCC® 43340™)

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: *Methanosarcina mazei* (ATCC® 43340™)

Description

Designation: C16 [DSM 3318]

Deposited Name: *Methanococcus frisius* Blotevogel et al.

Propagation

Medium

ATCC® Medium 1439: Methanogenium medium

Growth Conditions

Temperature: 37.0°C

Atmosphere: Under a gas mixture of 80% H₂, 20% CO₂

Propagation Procedure

1. Sterilize the top of the Balch tube (see below) by spraying it with 70% ethanol and then flame the top.
2. If needed exchange the gas in the test tube for 80% H₂ 20% CO₂; best results are obtained if the tube is pressurized to approximately 1.52 bar.
3. If the medium is pink (see discussion about resazurin) add 2.0 ml of reducing agent (1.5% sodium sulfide, stock solution) per 100 ml of medium. Let the medium sit at room temperature for 10 to 20 minutes, until the resazurin becomes colorless, before inoculating.
4. When the Balch tube is ready to inoculate, thaw the frozen vial at room temperature under a gentle stream of oxygen free gas.
5. For inoculation, use a 1.0 ml syringe tipped with 22 gauge needle, withdraw the cell suspension from the vial and transfer it to the broth. Plate 0.1 ml of the inoculated culture onto a non-selective medium and incubate aerobically at 37°C. Use 0.1 ml of the inoculated culture to inoculate a nonselective aerobic broth. Incubate the inoculated tubes at 37°C.
6. Growth should be detected in the #1439 broth within 24 to 48 hours. There should be no growth detected on the aerobic plate or broth.

ANAEROBIC CONDITIONS:

- a. Balch tube refers to a special type of test tube that is designed to be pressurized and is suited for anaerobic work. The Balch test tubes can be purchased from Bellco Glass (www.bellcoglass.com; stock no. 2048-00150).
- 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.
- d. Syringes can be made anaerobic by one of two methods. 1. Displace the dead space in the syringe with a sterile

Notes

Cells appear as large single cocci. Cells autofluoresce when viewed using a UV filter cube on an epifluorescence microscope.

This culture will also grow in Thioglycollate, ATCC Medium #177.

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.

ATCC Warranty

The viability of ATCC® products is warranted for 30 days from the date of shipment, and is valid only if the product is stored and cultured according to the information included on this product information sheet. ATCC lists the media formulation that has been found to be effective for this

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strain. While other, unspecified media may also produce satisfactory results, a change in media or the absence of an additive from the ATCC recommended media may affect recovery, growth and/or function of this strain. If an alternative medium formulation is used, the ATCC warranty for viability is no longer valid.

Disclaimers

This product is intended for laboratory research purposes only. It is not intended for use in humans.

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Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at www.atcc.org

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

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