ATCC

Product Sheet

Methanosarcina mazei (ATCC® BAA-2020™)

Please read this FIRST

Storage Temp.
Frozen: -80°C or colder
Freeze-Dried: 2°C to 8°C
Live Culture: See Propagation Section

Biosafety Level
1

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® BAA-2020™)

American Type Culture Collection
PO Box 1549
Manassas, VA 20108 USA
www.atcc.org

800.638.6597 or 703.365.2700
Fax: 703.365.2750
Email: Tech@atcc.org

Or contact your local distributor

Description

Designation: FA9604c [OCM 665]

Propagation

Medium
ATCC® Medium 2467: MS - OCM Base Medium

Growth Conditions
Temperature: 37°C
Atmosphere: Anaerobic, 80% H_2-20% CO_2

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% H_2-20% CO_2; do not go above 5 PSI.
3. If the medium is pink (see discussion about resazurin) add 0.1 mL reducing agent (1.5% $Na_2S{\cdot}9H_2O$, stock solution) per each 5-6 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, open the vial thaw in an oxygen free environment.
5. For inoculation, use a 1.0 mL syringe tipped with 22 gauge needle. Make the syringe anaerobic (see discussion below) and draw the thawed cell suspension up into the syringe. Transfer the cell suspension into a tube of pre reduced #2467 broth and incubate at 30°C. Secondary tubs can be inoculated by transferring 0.5 mL of the primary tube. Plate 0.1 mL of the inoculated culture onto a non-selective medium and incubate aerobically at 30°C. Inoculate a nonselective anaerobic and aerobic broth and incubate at 30°C
6. Growth should be detected in the #2467 broth within 5 to 7 days. The gas in the head space of the Balch tube should be exchanged for fresh 80% H_2-20% CO_2 every 2 to 3 days. There should be no growth detected on the aerobic plate. There should be no growth in the nonselective aerobic or anaerobic 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 form 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.
d. Common reducing agents are sodium sulfide, cysteine, and coenzyme-M (See below).
e. Syringes can be made anaerobic by one of two methods.
    1. Displace the dead space in the syringe with a sterile oxygen-free gas.
    2. Displace the dead space in the syringe with a reducing agent.

Notes

If acetate or methanol is included in the medium then the 80% Hydrogen-20% Carbon dioxide gas does not need to be exchanged.
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.

ATCC Warranty

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ATCC® products are warranted for 30 days from the date of shipment, and this warranty is valid only if the product is stored and handled according to the information included on this product information sheet. If the ATCC® product is a living cell or microorganism, ATCC lists the media formulation that has been found to be effective for this product. 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 product. 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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