



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**

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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](http://www.atcc.org)

800.638.6597 or 703.365.2700  
Fax: 703.365.2750  
Email: [Tech@atcc.org](mailto: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<sub>2</sub>-20% CO<sub>2</sub>

### 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<sub>2</sub>-20% CO<sub>2</sub>; 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<sub>2</sub>S·9H<sub>2</sub>O, 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<sub>2</sub>-20% CO<sub>2</sub> 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 from Bellco glass ([www.bellcoglass.com](http://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](http://www.atcc.org).

## References

References and other information relating to this product are available online at [www.atcc.org](http://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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**Disclaimers**

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Additional information on this culture is available on the ATCC web site at [www.atcc.org](http://www.atcc.org).  
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