



Product Sheet

# *Methanosarcina vacuolata* (ATCC® 35090™)

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 vacuolata* (ATCC® 35090™)

## Description

**Designation:** DSM 1232 [Z-761]

**Deposited Name:** *Methanosarcina vacuolata* Zhilina and Zavarzin

## Propagation

### Medium

ATCC® Medium 1043: Methanosarcina medium

### Growth Conditions

**Temperature:** 37.0°C

**Atmosphere:** Under a gas mixture of 80% H<sub>2</sub>, 20% CO<sub>2</sub>

### Propagation Procedure

1. Sterilize the top of the Hungate test tube with 70% ethanol.
2. Exchange gas in the Hungate test tube for 80% H<sub>2</sub> - 20% CO<sub>2</sub>.
3. If the medium is oxidized (see discussion about resazurin below) all 0.1 ml of reducing agent (see above) to the medium and let the medium sit for 30 minutes before inoculating.
4. When the Hungate test tube is ready to be inoculated, place the frozen LN<sub>2</sub> vial under a stream of oxygen free gas and thaw at room temperature.
5. Using a syringe, in which the dead space has been filled with an anaerobic gas mixture or reducing agent (see below), withdraw the cell suspension from vial and transfer to a single tube (5 to 6 ml) of the recommended 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. 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

Using the syringe transfer method, you must make the transfer as quickly as possible. Sometimes during transfer the medium will oxidize and turn pink (due to resazurin), however it may reduce itself back to the clear broth color during incubation. If the color does not change back, anaerobic conditions were not met and the culture will not grow.

Cells occur in packets of two, four, or eight large cocci. No motility has been detected.

## 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.

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longer valid.

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### **Disclaimers**

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

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