



Product Sheet

# *Methanoculleus marisnigri* (ATCC® 35101™)

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: *Methanoculleus marisnigri* (ATCC® 35101™)

## Description

**Designation:** DSM 1498 [JR1]

**Deposited Name:** *Methanogenium marisnigri* Romesser et al.

## Propagation

### Medium

ATCC® Medium 1343: General salts medium for estuarine methanogens

### Growth Conditions

**Temperature:** 37.0°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. Exchange the gas in the test tube for 80% H<sub>2</sub>-20% CO<sub>2</sub>; do not over pressurize (max of 5 psi).
3. If the medium is pink (see discussion about resazurin) add 2.0 ml of reducing agent (3% cysteine, 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. Open the frozen vial and immediately place the vial under a stream of sterile oxygen free gas, to maintain anaerobic conditions.
5. Using the same syringe, withdraw the cell suspension from the vial and transfer it to the Balch tube. Plate 0.1 ml of the inoculated culture onto a non-selective medium and incubate aerobically at 37°C. Add more 80% H<sub>2</sub>-20% CO<sub>2</sub> to the Balch tube(s) (Pressurize up to 20 psi) and incubate at 37°C.
6. Growth should be detected in the broth within 48-72 hours. No growth should be detected on the aerobic plate or broth.

### 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 up 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

Growth may take up to one week when the frozen culture is initially inoculated into fresh medium.  
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.

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Manassas, VA 20108 USA  
[www.atcc.org](http://www.atcc.org)

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Fax: 703.365.2750  
Email: [Tech@atcc.org](mailto:Tech@atcc.org)

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Biosafety Level  
1

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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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Manassas, VA 20108 USA  
[www.atcc.org](http://www.atcc.org)

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Fax: 703.365.2750  
Email: [Tech@atcc.org](mailto:Tech@atcc.org)

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