



Product Sheet

Methanoplanus limicola (ATCC® 35062™)

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: *Methanoplanus limicola* (ATCC® 35062™)

Description

Designation: DSM 2279 [M3]

Deposited Name: *Methanoplanus limicola* Wildgruber 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 by spraying it with 70% ethanol and then flaming the top.
2. If needed exchange the gas in the test tube for 80% H₂ 20% CO₂
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. After the Balch tube is ready to be inoculated let the frozen vial thaw at room temperature under a gentle stream of sterile oxygen free gas.
5. Using a 1.0 ml syringe tipped with 22 gauge needle, withdraw the cell suspension from the vial and transfer it to the broth and incubate at 37°C. Plate 0.1 ml of the inoculated culture onto a non-selective medium and incubate aerobically at 37°C.
6. Growth should be detected in the broth within 48 to 96 hours. No growth should be detected on the aerobic plate or broth.

ANAEROBIC CONDITIONS:

- 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.
- 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.
- Syringes can be made anaerobic by one of two methods.
 1. Displace the dead space in the syringe with a sterile
 2. Displace the dead space in the syringe with a reducing

Notes

It has been our experience the greater the headspace the better the growth. Therefore, 25 ml Balch (Hungate) tubes with 4-5 ml of media should be used.

It is also recommended that you pressurize the headspace with at least 20 psi to allow for good gas exchange. For good growth, gas the culture every day for 5 to 6 days.

The cells occur singly and in pairs. The cells appear as thin plates with sharp edges and in some cases there is branching with no septum formation.

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.

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Biosafety Level
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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.

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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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