ATCC

Product Sheet

Methanococcus maripaludis (ATCC® BAA-1333™)

Please read this FIRST

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: *Methanococcus maripaludis* (ATCC® BAA-1333™)

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
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Or contact your local distributor

Description

Designation: C5

Propagation

Medium
ATCC® Medium 1439: Methanogenium medium

Growth Conditions
Atmosphere: hydrogen (H2), 80%; carbon dioxide (CO2), 20%
Temperature: 37.0°C

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_2 20% CO_2, do not pressurize over 5psi. If the tubes are over pressurized (20 psi.), inoculating the tubes will prove difficult.
3. Prepare tubes for inoculation: If the medium is pink (see discussion about resazurin B) add 2.0 ml of reducing agent (5% Co-enzyme M stock solution) per 100 ml of medium. Let the medium sit at room temperature for at least 1 hour or until the resazurin becomes colorless, before inoculating.
4. Thaw the frozen vial under a gentle stream of anaerobic gas. Using an anaerobic (see E) 1.0 ml syringe tipped with 22-gauge needle, withdraw the cell suspension from the vial and transfer to the tube of ATCC #1439 medium. Transfer 0.5 ml of the inoculated culture to a second tube of ATCC medium #1439. Pressurize the culture tubes to 20 psi with 80% H_2 20% CO_2. Plate 0.1 ml of the inoculated culture onto a non-selective medium and incubate the plate aerobically at 37ºC. Incubate culture tubes at 37ºC.
5. Growth should be detected in the broth within 4 to 6 days. No growth should be detected on the aerobic plate.

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 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, titanium citrate and Co-enzyme M (see D).

D. We suggest adding the reducing agent to the medium at least one hour before the medium is to be inoculated. Co-enzyme M (mercaptoethanesulfonic acid) (100 X solution): *Dissolve 5.0 g in 100 ml of deionized water. Distribute into screw cap test tubes, 56 ml per tube and seal with rubber stoppers under N_2 gas. Autoclave to sterilize. Excess tubes can be stored at room temperature for up to 2 months. Co-enzyme M is a compound produced by many methanogens. Some methanogens are sensitive to stronger reducing agents such as sodium sulfide. Co-enzyme M is the standard reducing agent we use when working with methanogens.*

E. Syringes can be made anaerobic by one of two methods.

Notes

Cells occur singly and in pairs. The cells exhibited characteristic F420 autofluorescence by epifluorescence microscopy using a low wavelength filter set.
Always use freshly prepared anaerobic medium. If there is any question about the anaerobic condition of the medium, the it can be reduced with the addition of 5.0% Co-enzyem M (2.0 ml per 100 ml of medium).

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

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for Health.

ATCC Warranty

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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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