Methanogenium cariaci Romesser et al. emend. Maestrojuan et al.

BAA-2058™

Description

Strain designation: JR1
Type strain: No

Storage Conditions

Product format: Frozen
Storage conditions: -80°C or colder

Intended Use

This product is intended for laboratory research use only. It is not intended for any animal or human therapeutic use, any human or animal consumption, or any diagnostic use.

BSL 1

ATCC determines the biosafety level of a material based on our risk assessment as guided by the current edition of *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, U.S. Department of Health and Human Services. It is your responsibility to understand the hazards associated with the material per your organization's policies and procedures as well as any other applicable regulations as enforced by your local or national agencies.

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ATCC highly recommends that appropriate personal protective equipment is always used when handling vials. For cultures that require storage in liquid nitrogen, it is important to note that some vials may leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vial exploding or blowing off its cap with dangerous force creating flying debris. Unless necessary, ATCC recommends that these cultures be stored in the vapor phase of liquid nitrogen rather than submersed in liquid nitrogen.

Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at www.atcc.org.

Growth Conditions

Medium:
ATCC Medium 2467: MS - OCM Base Medium
Temperature: 30°C
Atmosphere: 80% H_2, 20% CO_2

Handling Procedures

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_2 -20% CO_2; do not go above 5 PSI.
3. If the medium is pink (see discussion about resazurin) add 0.1 mL reducing agent (1.5% $Na_2S{\cdot}9H_2O$, 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_2 -20% CO_2 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:

1. 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 form Bellco glass (www.bellcoglass.com; stock no. 2048-00150).
2. 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.
3. To obtain a fully reduced medium, it is necessary that the medium be anoxic and that a reducing agent be added.
4. Common reducing agents are sodium sulfide, cysteine, and coenzyme-M (See below).
5. Syringes can be made anaerobic by one of two methods. 1
    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%

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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.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Methanogenium cariaci* Romesser et al. emend. Maestrojuan et al. (ATCC BAA-2058)

References

References and other information relating to this material are available at www.atcc.org.

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