Methanosarcina barkeri Schnellen

BAA-2329™

Description

Strain designation: Fusaro
Deposited As: *Methanosarcina barkeri* Schnellen

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.

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 (5 psi)

Handling Procedures

1. Sterilize the top of the Balch tube by spraying it with 70% ethanol and then flaming the top. Add trimethyamine to the broth to reach a level of 0.1 M.
2. Exchange the gas in the test tube for 80% H_2 - 20% CO_2.
3. If the medium is pink (see discussion about resazurin), add 0.2 mL Coenzyme M (5% stock solution) per 10 mL of medium. Let the medium sit at room temperature for at least 24 hours until the resazurin becomes colorless before inoculating. If the medium is a light blue or purple, it is not completely

anaerobic.

4. When the Balch tube is ready to inoculate, thaw the frozen vial at room temperature under a gentle stream of 80% H_2 - 20% CO_2 gas. Add 0.1 M trimethylamine.
5. For inoculation, use an anaerobic 1.0 mL syringe tipped with 22gauge needle, withdraw the cell suspension from the vial and transfer it to the broth. Additional tubes of #2467 can be inoculated by transferring 0.5 mL of the primary broth using good anaerobic technique. Plate 0.1 mL of the inoculated culture onto a non-selective medium. Exchange the gas in the Balch tubes again after inoculation.
6. Incubate one plate aerobically and another anaerobically at 37°C. Incubate the anaerobic tube(s) at 30°C.
7. Initial growth should be detected in the #2467 broth within 48 to 72 hours. Optimal growth will be observed at 5 to 7 days. More than one transfer may be required to achieve light turbidity. There should be no growth detected on either plate.

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. 204800150).
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. Common reducing agents are sodium sulfide, 3% cysteine, or 1.5% sodium sulfide (stock solutions), Add 0.2 mL of reducing agent for each 10 mL of medium. The depositor used sodium sulfide as a reducing agent, however, Coenzyme M is the standard reducing agent used at ATCC when working with methanogens due to their sensitivity.
4. Syringes can be made anaerobic by one of two methods.
    a. Displace the dead space in the syringe with a sterile oxygen-free gas.
    b. Displace the dead space in the syringe with a reducing agent.

Notes

0.1 M trimethylamine is necessary to sustain growth of this organism. Alternatively, the pressure of the hydrogen gas atmosphere in the tube can be increased to 20 psi, and the culture will grow by using the hydrogen gas.
Culture is inhibited by both O_2 and N_2 and needs to be propagated in the correct gas mixture.

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: *Methanosarcina barkeri* Schnellen (ATCC BAA-2329)

References

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

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