Methanosarcina barkeri Schnellen

BAA-2362™

Description

Previously sold as ATCC® 29786™.

Strain designation: Strain DSM 805: Strain 3

Deposited As: *Methanosarcina barkeri* Schnellen

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.

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 2825: Modified 1043 Broth: Methanosarcina Medium
Temperature: 30°C
Atmosphere: 80% H_2, 20% CO_2

Handling Procedures

1. Sterilize the top of the Hungate test tube with 70% ethanol. Using a sterile syringe, add 0.1 mL of a 1 M solution of trimethylamine for each 10 mL of medium.
2. Exchange gas in the Hungate test tube for 5 psi of 80% H_2 - 20% CO_2. Do not over pressurize since this will make it difficult to inoculate the tube(s). This organism is able to use substrates other than 80% H_2 - 20% CO_2 including

trimetheylamine and acetate (see notes).

3. If the medium is oxidized (see discussion about resazurin below) all 0.1 mL of reducing agent (see above) to the medium and let the medium sit for 30 minutes before inoculating.
4. When the Hungate test tube is ready to be inoculated, place the frozen LN_2 vial under a stream of oxygen free gas and thaw at room temperature.
5. Using a syringe, in which the dead space has been filled with an anaerobic gas mixture or reducing agent (see below), withdraw the cell suspension from vial and transfer to a single tube (5 to 6 mL) of the recommended broth.

ANAEROBIC CONDITIONS

a. 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 from Bellco Glass (www.bellcoglass.com; stock no. 2048-00150).
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.
d. Syringes can be made anaerobic by one of two methods.
    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

Within 48 to 72 hours, growth should be evident by turbidity that settles to the bottom of the test tube. **This strain is found to grow very well with trimethylamine as a substrate.**

Using the syringe transfer method, make the transfer as quickly as possible. Sometimes during transfer, the medium will oxidize and turn pink (due to resazurin), however it may reduce itself back to the clear broth color during incubation. If the color does not change back, anaerobic conditions were not met and the culture will not grow.

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-2362)

References

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

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