ATCC

Product Sheet

Methanosarcina barkeri (ATCC® 43569™)

Please read this FIRST

Storage Temp.
Frozen: -80°C or colder
Freeze-Dried: 2°C to 8°C
Live Culture: See Propagation Section

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: *Methanosarcina barkeri* (ATCC® 43569™)

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
Email: Tech@atcc.org

Or contact your local distributor

Description

Designation: [ATCC 51582, DSM 800, MS, OCM 38]
Deposited Name: *Methanosarcina barkeri* Schnellen
Product Description: Type strain

Propagation

Medium
ATCC® Medium 2889: SAB Broth

Growth Conditions
Temperature: 37°C
Atmosphere: Anaerobic gas mixture, 80% H_2-20% CO_2

Propagation Procedure

1. Pre-reduce media in the following manner: Add trimethylamine to a final concentration of 20 mM. Exchange gas in the headspace of the Baltch tube of media with 80% H_2-20% CO_2 gas at 5 psi. The medium must be colorless before inoculating. If a hint of resazurin is noticeable (see note below), add 0.1 mL reducing agent.
2. Open thawed vial according to enclosed instructions or visit www.atcc.org for instructions.
3. Under anaerobic conditions aseptically transfer the entire contents to a 5-6 mL Baltch tube of #2889 broth. Additional test tubes can be inoculated by transferring 0.5 mL of the primary broth tube to these secondary broth tubes. Best practice dictates the use of pre-reduced media.
4. Incubate in an anaerobic atmosphere at 37°C for 7 days. Incubate one agar plate aerobically at 37°C to check for contamination.

ANAEROBIC CONDITIONS:

a. 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.
b. 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.
c. 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.

Anaerobic conditions for incubation may be obtained by any of the following:

- Loose screw caps on test tubes in an anaerobic chamber
- Loose screw caps on test tubes in an activated anaerobic gas pack jar
- Use of sterile butyl rubber stoppers on test tubes so that an anaerobic gas headspace is retained

Notes

This organism requires the addition of 20 mM trimethylamine to the broth for best growth.
Culture may have to be established in the primary broth before subcultures can be made.

Reducing agents:
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, 5–6 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.*

Sodium sulfide (100 X solution): *Dissolve 1.5 g in 100 mL of distilled water. Distribute into screw cap test tubes, 5–6 mL per tube, and seal with Hungate stoppers. Autoclave to sterilize. Excess tubes can be stored frozen for up to 6 months. Once thawed a tube of sodium sulfide should not be used for more than a week. CAUTON: if sodium sulfide comes into contact with a strong acid, hydrogen sulfide (H_2S), a very toxic gas, is liberated immediately.*
Cysteine (100X solution): *Dissolve 3.0 g in 100 mL of distilled water. Distribute into screw cap test tubes, 5–6 mL per tube, and seal with Hungate stoppers. Autoclave to sterilize. Excess tubes can be stored frozen*

ATCC

Product Sheet

Methanosarcina barkeri (ATCC® 43569™)

Please read this FIRST

Storage Temp.
Frozen: -80°C or colder
Freeze-Dried: 2°C to 8°C
Live Culture: See Propagation Section

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: *Methanosarcina barkeri* (ATCC® 43569™)

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
Email: Tech@atcc.org

Or contact your local distributor

for up to 6 months. Once thawed, a tube of cysteine should not be used for more than a week.

Substrates: Useful Information:
Some methanogens are able to utilize substrates other than H_2-CO_2, such as acetate, propionate, methanol, etc. These substrates can be added directly to the tubed medium, making it possible to use the same medium for more than one organism. We suggest making up anaerobic stock solutions at 100X. Some of these substrates (organic acids) need to be neutralized with sodium hydroxide.
Amount per 100 mL for 1M solution:

- Acetic acid: 5.7 mL
- Propionic acid: 7.5 mL
- Butyric acid: 9.2 mL
- Formic acid (tech, 90%): 4.2 mL
- Methanol (100%): 4.0 mL

WARNING: EXPLOSION HAZARD:
Methanogens that grow on methanol produce methane gas resulting in over pressurization of growth vessels (3). This creates a potential explosion hazard. We recommend growing cultures in pressure-resistant Balch tubes* to reduce this risk. The cultures should be vented regularly to reduce the gas and prevent overpressure. If it is necessary to grow larger batches of methanol-utilizing methanogens in sealed serum vials, extra caution should be taken. Typically, 3 moles of methane are produced from one mole of methanol. Always wear protective eye wear when working with methanogens growing in tubes or bottles.

Additional information on this culture is available on the ATCC® web site at www.atcc.org.

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.

ATCC Warranty

ATCC® products are warranted for 30 days from the date of shipment, and this warranty is valid only if the product is stored and handled according to the information included on this product information sheet. If the ATCC® product is a living cell or microorganism, ATCC lists the media formulation that has been found to be 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.

Disclaimers

This product is intended for laboratory research purposes only. It is not intended for use in humans.
While ATCC uses reasonable efforts to include accurate and up-to-date information on this product sheet, ATCC makes no warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. ATCC does not warrant that such information has been confirmed to be accurate.
This product is sent with the condition that you are responsible for its safe storage, handling, and use. ATCC is not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to insure authenticity and reliability of materials on deposit, ATCC is not liable for damages arising from the misidentification or misrepresentation of such materials.

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.