Methanosarcina barkeri Schnellen

43569™

Description

Type strain
Strain designation: [ATCC 51582, DSM 800, MS, OCM 38]
Deposited As: *Methanosarcina barkeri* Schnellen
Type strain: Yes

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 2889: SAB Broth
Temperature: 37°C
Atmosphere: 80% H_2, 20% CO_2

Handling Procedures

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 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.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Methanosarcina barkeri* Schnellen (ATCC 43569)

References

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

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