Methanothermobacter marburgensis Wasserfallen et al.

BAA-927™

Description

Type strain.
Strain designation: OCM 82 [DSM 2133]
Deposited As: *Methanothermobacter marburgensis* Wasserfallen et al.
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 2487: MS-OCM Base Medium with 43 mM NaCl and 5 mM sodium acetate
Temperature: 55°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 flame the top.
2. If needed 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 0.1 mL $Na_2S{\cdot}9H_2O$

(1.5% sodium sulfide, stock solution) per 10 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, thaw the frozen vial at room temperature under a gentle stream of oxygen-free gas.
5. For inoculation, use an anaerobic (see c below) 1.0 mL syringe tipped with 22-gauge needle, withdraw the cell suspension from the vial and transfer it to the broth. Plate 0.1 mL of the inoculated culture onto a non-selective medium and incubate aerobically at 37ºC. Use 0.1 mL of the inoculated culture to inoculate a nonselective aerobic broth and an additional tube of #2487 broth. Incubate the non-selective aerobic broth tubes at 37ºC. Incubate the anaerobic tube at 55ºC.
6. Growth should be detected in the #2487 broth within 24 to 48 hours. There should be no growth detected on the aerobic plate or in the aerobic broth.

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.

Notes

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 1-M 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 (a solute) 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* (*see below*) 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: *Methanothermobacter marburgensis* Wasserfallen et al. (ATCC BAA-927)

References

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

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Revision

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