Methanolobus zinderi Doerfert et al. 2009

BAA-1601™

Description

Strain designation: SD1 [DSM 21339]
Deposited As: *Methanolobus zinderii*
Type strain: Yes

Storage Conditions

Product format: Frozen

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 1355: Methanosarcina acetovorans medium
Temperature: 35-37°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 flaming the top.

2. Exchange the gas in the test tube for 80% H_2 20% CO_2; do not pressurize over 5psi. If the tubes are over pressurized (20 psi), inoculating the tubes will prove difficult.

3. Prepare tubes for inoculation: If the medium is pink (see discussion about

resazurin B) add 2.0 ml of reducing agent (5% Co-enzyme M stock solution) per 100 ml of medium. Let the medium sit at room temperature for at least 1 hour or until the resazurin becomes colorless, before inoculating.

4. Thaw the frozen vial under a gentle stream of anaerobic gas. Using an anaerobic (see E) 1.0 ml syringe tipped with 22-gauge needle, withdraw the cell suspension from the vial and transfer to the tube of ATCC® #1355 medium. Transfer 0.5 ml of the inoculated culture to a second tube of ATCC® medium #1355. Plate 0.1 ml of the inoculated culture onto a non-selective medium and incubate the plate aerobically at 35-37ºC. Incubate culture tubes at 37ºC.

5. Growth should be detected in the broth within 4 to 6 days. No growth should be detected on the aerobic plate.

ANAEROBIC CONDITIONS:

A. Balch tubes (available from Bellco Glass, Vineland, NJ; are specially designed for anaerobic work and use an aluminum crimp cap to hold a rubber stopper in place. Needles can easily be inserted through the stopper, and the tubes can be pressurized to 2 atm. Alternatively, serum vials may be used, or screw cap tubes with butyl rubber stoppers, in the latter case the stopper may be removed and the tube placed under a cannula system that dispenses sterile, oxygen free gas for addition of reducing agents or inoculation.

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, titanium citrate and Co-enzyme M (see D).

D. 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, 56 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.

E. Syringes can be made anaerobic by one of two methods.

Notes

Cells occur singly and in pairs. The cells exhibit characteristic F420 autofluorescence by epifluorescence microscopy using a low wavelength filter set.

Always use freshly prepared anaerobic medium. If there is any question about the anaerobic condition of the medium, it can be reduced with the addition of 5.0% Co-enzyem M (2.0 ml per 100 ml of medium).

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Methanolobus zinderi* Doerfert et al. 2009 (ATCC BAA-1601)

References

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

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Contact Information

ATCC
10801 University Boulevard
Manassas, VA 20110-2209
USA
US telephone: 800-638-6597
Worldwide telephone: +1-703-365-2700
Email: tech@atcc.org or contact your local distributor