Designation: CR-1 [DSM 2373]

Deposited Name: Methanogenium thermophilicum Rivard and Smith

Medium
ATCC® Medium 1442: Methanoculleus thermophilicus medium

Growth Conditions
Temperature: 55.0°C
Atmosphere: Under a gas mixture of 80% H₂, 20% CO₂

Propagation Procedure
1. Sterilize the top of the Balch tube by spraying it with 70% ethanol and then flaming the top.
2. If needed exchange the gas in the test tube for 80% H₂, 20% CO₂.
3. If the medium is pink (see discussion about resazurin) add 2.0 ml of reducing agent (3% cysteine, stock solution) per 100 ml of medium. Let the medium sit at room temperature for 10 to 20 minutes, until the resazurin becomes colorless, before inoculating.
4. After the Balch tube is ready to be inoculated let the frozen vial thaw at room temperature under a gentle stream of sterile oxygen free gas.
5. Using a 1.0 ml syringe tipped with 22-gauge needle, withdraw the cell suspension from the vial and transfer it to the broth and incubate at 55°C. Plate 0.1 ml of the inoculated culture onto a non-selective medium and incubate aerobically at 37°C.
6. Within 48 to 72 hours, growth should be evident by turbidity that settles to the bottom of the test tube. No growth should occur on the blood agar plate incubated aerobically.

Anaerobic Conditions:
· 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.
· 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.
· Syringes can be made anaerobic by one of two methods.
  1. Displace the dead space in the syringe with a sterile gas stream.

Notes
Cells are Gram negative, irregular cocci occurring singly or in pairs. No motility has been detected.
This strain is able to utilize formate or hydrogen and carbon dioxide.

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.

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