Methanococcus maripaludis Jones et al.

BAA-2049™

Description

Strain designation: LL [OCM 803]
Type strain: No

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.

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.*Balch tube refers to a special type of test tube that is designed to be pressurized and suited for anaerobic work.

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 2469: MSH Medium
Temperature: 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. If needed, exchange the gas in the test tube for 80% H_2 20% CO_2. Do not over pressurize (5 psi until the culture has been inoculated).

3. If the medium is pink (see discussion about resazurin), add 0.2 ml reducing agent (see note Reducing agents below) per 10 ml of medium. Let the medium sit at room temperature for minimum of 30 to 40 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. Additional tubes of MSH can be inoculated by transferring 0.5 ml of the primary broth using good anaerobic technique. Plate 0.1 ml of the inoculated culture onto a non-selective medium and incubate aerobically at 37°C. Use 0.1 ml of the inoculated culture to inoculate a nonselective aerobic broth. Incubate the non-selective aerobic broth tubes at 37°C. Increase the head space pressure to 20 psi and incubate the anaerobic tubes at 37°C.

6. Growth should be detected in the MSH broth within 24 to 48 hours. There should be no growth detected on the aerobic plate or in the aerobic broth.

ANAEROBIC CONDITIONS:

a. Balch tube refers to a special type of test tube that is designed to be pressurized and is suited for anaerobic work. The Balch test tubes can be purchased form Bellco glass (www.bellcoglass.com; stock no. 2048-00150).

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, 3% cysteine, or 1.5% sodium sulfide (stock solutions), Add 0.1 ml of reducing agent for each 10 ml of medium. For BAA-2049?, sodium sulfide worked well as a reducing agent.

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

Cells are sarcina in shape and occur singly, in pairs, and in clumps; growth settles to the bottom of the tube. No growth should occur on non-selective media either aerobically or anaerobically.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Methanococcus maripaludis* Jones et al. (ATCC BAA-2049)

References

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

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Revision

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