Methanococcus vannielii Stadtman and Barker

35089™

Description

Strain designation: DSM 1224 [SB]
Deposited As: *Methanococcus vannielii* Stadtman and Barker
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 2467: MS - OCM Base 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 pressurize above 5 psi. If the tube is over pressurized, it will be difficult to inoculate.

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 30 minutes (or until the resazurin becomes colorless) before inoculating.

4. Open the frozen vial and immediately place the vial under a stream of sterile anaerobic gas, to maintain anaerobic conditions. Wait for vial contents to thaw.

5. Using an anaerobic syringe (see d below), withdraw the cell suspension from the vial and transfer it to the primary Balch tube. Plate 0.1 ml of the inoculated culture onto a non-selective medium and incubate aerobically at 37ºC. Additional tubes can be inoculated by transferring 0.5 ml of the primary tube to secondary tube(s). Again, use good anaerobic technique. Once inoculated the Balch tubes should be pressurized up to 20 psi with 80% H_2-20% CO_2.

6. Growth should be detected in the broth within 48-72 hours. No growth should be detected on the aerobic plate or broth.

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 up to 20 psi. 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, and titanium citrate.

Notes

Growth may take up to one week when the frozen culture is initially inoculated into fresh medium. The broth culture appears as a smooth pellicle and growth throughout the tube.

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: *Methanococcus vannielii* Stadtman and Barker (ATCC 35089)

References

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

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