Methanomicrobiales

43114™

Description

Strain designation: DE
Deposited As: *Methanogenium wolfei*
Type strain: No

Storage Conditions

Product format: Freeze-dried
Storage conditions: 2°C to 8°C

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 1340: MS medium for methanogens
Temperature: 45°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. It has been our experience the greater the headspace the better the growth. Therefore, 25 mL Balch (Hungate) tubes with 4-5 mL of media should be used. It is also recommended that you pressurize the headspace with at least 20 psi to allow for good gas exchange. For good growth, gas the culture every day for 3 to 4 days.

3. If the medium is pink (see discussion about resazurin), add 2.0 mL of reducing agent (Na_2S) 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 45°C. Plate 0.1 mL of the inoculated culture onto a nonselective medium and incubate aerobically at 45°C.
6. Growth should be detected in the broth within one week. No growth should be detected on the aerobic plate or broth.

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.

Notes

It has been our experience the greater the headspace the better the growth. Therefore, 25 mL Balch (Hungate) tubes with 4-5 mL of media should be used.
It is also recommended that you pressurize the headspace with at least 20 psi to allow for good gas exchange. For good growth, gas the culture every day for 3 to 4 days.
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: Methanomicrobiales (ATCC 43114)

References

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

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