



***Methanobacterium alcaliphilum* Worakit et al.**

43379™

Description

Strain designation: WeN4 [DSM 3387]

Deposited As: *Methanobacterium alcaliphilum* Worakit et al.

Type strain: Yes

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.

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% H₂, 20% CO₂ (20 psi)

Handling Procedures

1. Sterilize the top of the Balch tube by spraying it with 70% ethanol and then flame the top.
2. If needed exchange the gas in the test tube for 80% H₂–20% CO₂ (20 psi).
3. If the medium is pink (see discussion about resazurin) add 0.1 ml Co-enzyme M (5% stock solution, see below) per 10 ml of medium. Let the medium sit at room temperature for a minimum of 30 minutes or up to 24 hours. The

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resazurin must be 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. 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 and an additional tube of #2467 broth. Incubate the non-selective aerobic broth tubes at 37°C. Incubate the anaerobic tube at 37°C.
6. Growth should be detected in the #2467 broth within 10 days. The gas in the culture tubes should be exchanged every 2 to 3 days to promote growth. There should be no growth detected on the aerobic plate or in the aerobic broth.

ANAEROBIC CONDITIONS:

- a. 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.
- 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, cysteine, dithiothreitol, and titanium citrate.
- 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

When preparing ATCC Medium #2467: MS – OCM Base Medium for this strain, adjust pH to 8.0-8.5 (10% stock solution of Na₂CO₃ can be used).

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: *Methanobacterium alcaliphilum* Worakit et al. (ATCC 43379)

References

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

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Revision

This information on this document was last updated on 2023-02-26

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