



# ***Methanothermococcus thermolithotrophicus*** (Huber et al.) Whitman

35097™

## **Description**

**Strain designation:** DSM 2095 [JCM 10549, OCM 138, SN-1]

**Deposited As:** *Methanococcus thermolithotrophicus* Huber et al.

**Type strain:** Yes

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## **Storage Conditions**

**Product format:** Frozen

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## **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.

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## **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.

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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 submerged 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 submerged in liquid nitrogen.

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### **Certificate of Analysis**

For batch-specific test results, refer to the applicable certificate of analysis that can be found at [www.atcc.org](http://www.atcc.org).

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### **Growth Conditions**

**Medium:**

ATCC Medium 1439: Methanogenium medium

**Temperature:** 60°C

**Atmosphere:** 80% H<sub>2</sub>, 20% CO<sub>2</sub>

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### **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<sub>2</sub> 20% CO<sub>2</sub>.
- 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**

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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 60°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

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**Notes**

Using the syringe transfer method, you must make the transfer as quickly as possible. Sometimes during transfer the medium will oxidize and turn pink (due to resazurin), however it may reduce itself back to the clear broth color during incubation. If the color does not change back, anaerobic conditions were not met and the culture will not grow.

Cells are Gram negative irregular cocci that occur singly and in pairs and are

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highly motile. This strain is able to utilize formate or hydrogen and carbon dioxide.

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### **Material Citation**

If use of this material results in a scientific publication, please cite the material in the following manner: *Methanothermococcus thermolithotrophicus* (Huber et al.) Whitman (ATCC 35097)

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### **References**

References and other information relating to this material are available at [www.atcc.org](http://www.atcc.org).

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**Revision**

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### **Contact Information**

ATCC

10801 University Boulevard

Manassas, VA 20110-2209

USA

US telephone: 800-638-6597

Worldwide telephone: +1-703-365-2700

Email: [tech@atcc.org](mailto:tech@atcc.org) or contact your local distributor

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