

700657<sup>TM</sup>

### Description

Strain designation: DSM 11074 [A8p]

**Deposited As:** Methanobacterium subterraneum Kotelnikova et al.

Type strain: Yes

### Storage Conditions

Product format: Freeze-dried

#### 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<sub>1</sub>

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 1892: Methanobacterium medium (DSM 119)

Temperature: 37°C

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

## Handling Procedures

- 1. Sterilize the top of the Hungate test tube with 70% ethanol.
- 2. Exchange gas in the Hungate test tube for  $80\% H_2 20\% CO_2$ .
- 3. If the medium is oxidized (see discussion about resazurin below) add 0.1 ml of reducing agent (1.5% Na<sub>2</sub>S·9H<sub>2</sub>O) to the medium and let the medium sit for 30 minutes before inoculating.



- 4. When the Hungate test tube is ready to be inoculated place the frozen LN<sub>2</sub> vial under a stream of oxygen free gas and thaw at room temperature.
- 5. Using a syringe, in which the dead space has been filled with an anaerobic gas mixture or reducing agent (*see below*), withdraw the cell suspension from vial and transfer to a single tube (5 to 6 ml) of the recommended broth.
- 6. An aerobic blood plate may be streaked to check for purity.
- 7. Incubate tubes under anaerobic conditions at 37°C. Incubate blood plate aerobically at 37°C.
- 8. Within 7 to 10 days, 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.

#### ANAFROBIC 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 to 2 atm. 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.
- D. Syringes can be made anaerobic by one of two methods.
  - 1. Displace the dead space in the syringe with a sterile
- 2. Displace the dead space in the syringe with a



#### Notes

Always use freshly prepared pre-reduced media or pre-reduced media that has been previously prepared but stored under anaerobic conditions. Resazurin in the media is a color indicator for anaerobic conditions. Observance of pink color in medium before use or during incubation shows anaerobic conditions have not been met and oxidation has occurred.

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 are not met and the culture will not grow.

#### **Material Citation**

If use of this material results in a scientific publication, please cite the material in the following manner: *Methanobacterium subterraneum* Kotelnikova et al. (ATCC 700657)

#### References

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

## Warranty

The product is provided 'AS IS' and the viability of ATCC® products is warranted for 30 days from the date of shipment, provided that the customer has stored and handled the product according to the information included on the product information sheet, website, and Certificate of Analysis. For living cultures, ATCC lists the media formulation and reagents that have been found to be effective for the product. While other unspecified media and reagents may also produce satisfactory results, a

change in the ATCC and/or depositor-recommended protocols may affect the recovery, growth, and/or function of the product. If an alternative medium formulation or reagent is used, the ATCC warranty for viability is no longer valid. Except as expressly set forth herein, no other warranties of any kind are provided, express or implied, including, but not limited to, any implied warranties of merchantability, fitness for a particular purpose, manufacture according to cGMP standards, typicality, safety, accuracy, and/or noninfringement.

#### **Disclaimers**

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. Any proposed commercial use is prohibited without a license from ATCC.

While ATCC uses reasonable efforts to include accurate and up-to-date information on this product sheet, ATCC makes no warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. ATCC does not warrant that such information has been confirmed to be accurate or complete and the customer bears the sole responsibility of confirming the accuracy and completeness of any such information.

This product is sent on the condition that the customer is responsible for and assumes all risk and responsibility in connection with the receipt, handling, storage, disposal, and use of the ATCC product including without limitation taking all appropriate safety and handling precautions to minimize health or environmental risk. As a condition of receiving the material, the customer agrees that any activity undertaken with the ATCC product and any progeny or modifications will be conducted in compliance with all applicable laws, regulations, and guidelines. This product is provided 'AS IS' with no representations or warranties whatsoever except as expressly set forth herein and in no event shall ATCC, its parents, subsidiaries, directors, officers, agents, employees, assigns, successors, and affiliates be liable for indirect, special, incidental, or consequential damages of any kind in connection with or arising out of the customer's use of the product. While reasonable effort is made to ensure authenticity and reliability of materials on deposit, ATCC is not liable for

damages arising from the misidentification or misrepresentation of such materials.

Please see the material transfer agreement (MTA) for further details regarding the use of this product. The MTA is available at www.atcc.org.

### Copyright and Trademark Information

© ATCC 2023. All rights reserved.

ATCC is a registered trademark of the American Type Culture Collection.

#### Revision

This information on this document was last updated on 2025-09-04

#### 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 or contact your local distributor

