



Methanobacterium subterraneum **Kotelnikova et al.**

700657™

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 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 1892: Methanobacterium medium (DSM 119)

Temperature: 37°C

Atmosphere: 80% H₂, 20% CO₂

Handling Procedures

1. Sterilize the top of the Hungate test tube with 70% ethanol.
2. Exchange gas in the Hungate test tube for 80% H₂ - 20% CO₂.
3. If the medium is oxidized (*see discussion about resazurin below*) add 0.1 ml of reducing agent (1.5% Na₂S·9H₂O) to the medium and let the medium sit for 30 minutes before inoculating.

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4. When the Hungate test tube is ready to be inoculated place the frozen LN₂ 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.

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 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
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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.

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