ATCC

Product Sheet

Methanobacterium subterraneum (ATCC® 700657™)

Please read this FIRST

Biosafety Level
1

Intended Use

This product is intended for research use only. It is not intended for any animal or human therapeutic or diagnostic use.

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: *Methanobacterium subterraneum* (ATCC® 700657™)

American Type Culture Collection
PO Box 1549
Manassas, VA 20108 USA
www.atcc.org

800.638.6597 or 703.365.2700
Fax: 703.365.2750
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Or contact your local distributor

Description

Designation: DSM 11074 [A8p]
Deposited Name: *Methanobacterium subterraneum* Kotelnikova et al.

Propagation

Medium
ATCC® Medium 1892: Methanobacterium medium (DSM 119)

Growth Conditions
Temperature: 37.0°C
Atmosphere: Under a gas mixture of 80% H2, 20% CO2

Propagation Procedure
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% $Na_2S{\cdot}9H_2O$) 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_2 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 37ºC. Incubate blood plate aerobically at 37ºC.
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

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.

References

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

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the current publication of the *Biosafety in Microbiological and Biomedical Laboratories* from the U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes for Health.

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ATCC Warranty

ATCC® products are warranted for 30 days from the date of shipment, and this warranty is valid only if the product is stored and handled according to the information included on this product information sheet. If the ATCC® product is a living cell or microorganism, ATCC lists the media formulation that has been found to be effective for this product. While other, unspecified media may also produce satisfactory results, a change in media or the absence of an additive from the ATCC recommended media may affect recovery, growth and/or function of this product. If an alternative medium formulation is used, the ATCC warranty for viability is no longer valid.

Disclaimers

This product is intended for laboratory research purposes only. It is not intended for use in humans.
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.
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Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at www.atcc.org

Additional information on this culture is available on the ATCC web site at www.atcc.org.

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