ATCC

Product Sheet

Methanobrevibacter smithii (ATCC® 35061™)

Please read this FIRST

Storage Temp.
Frozen: -80°C or colder
Freeze-Dried: 2°C to 8°C
Live Culture: See Propagation Section

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: *Methanobrevibacter smithii* (ATCC® 35061™)

American Type Culture Collection
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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 861 [PS]
Deposited Name: *Methanobrevibacter smithii* Balch and Wolfe
Product Description: Type strain. Genome sequenced strain.

Propagation

Medium
ATCC® Medium 1340: MS medium for methanogens

Growth Conditions
Temperature: 37°C
Atmosphere: Anaerobic gas mixture, 80% H_2-20% CO_2

Propagation Procedure

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 Balch tube for 80% H_2 - 20% CO_2. The Balch tube should not be pressurized over 5 psi until the culture has been inoculated. Once the culture has been inoculated, the pressure can be increased to 20 psi.
   - **Note:** When working with Balch tubes under high pressure, work away from open flames.
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 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 entire contents of the thawed vial and transfer it to the primary Balch tube and incubate at 37ºC. Additional Balch tubes can be inoculated by transferring 0.5 mL from the primary Balch tube to the additional Balch tubes. After the Balch tubes have been inoculated, increase the H_2 - CO_2 pressure to 20 psi. The headspace should be exhchanged for fresh H_2 - CO_2 every four to five days to get optimum growth.
   - **Note:** To check for culture purity, plate 0.1 mL of the inoculated culture onto a non-selective agar medium and incubate aerobically and anaerobically at 37ºC.
6. Growth should be detected in the broth within 5 to 6 days. No growth should be detected on the anaerobic or aerobic plates.

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
  2. Displace the dead space in the syringe with a reducing

Notes

It has been our experience that the greater the headspace, the better the growth. Therefore, 25 mL Balch (Hungate) tubes with 4-5 mL of media should be used.

It is also recommended that the headspace be pressurized with at least 20 psi to allow for good gas exchange. For optimum growth the head space should be exchanged for fresh H_2-CO_2 every day for 5 to 6 days.

This micoorganism does not grow on agar, only in broth. ATCC recommends a non-selective medium, such as a blood plate, to check for aerobic and anaerobic contaminants. No growth should occur on the aerobic or anaerobic blood plate, growth should only occur in anaerobic broth culture.

The cells occur singly and in pairs. The cells appear as lancet-shaped rods.

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

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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.

ATCC Warranty

The viability of ATCC® products is warranted for 30 days from the date of shipment, and is valid only if the product is stored and cultured according to the information included on this product information sheet. ATCC lists the media formulation that has been found to be effective for this strain. 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 strain. 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.

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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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