

***Methanobacterium espanolae* Patel et al.**

BAA-1073TM

Description

Strain designation: OCM 178 [GP9, NRC 5912]

Deposited As: *Methanobacterium espanolae* Patel et al.

Type strain: Yes

Storage Conditions

Product format: Frozen

Storage conditions: -80°C or colder

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.



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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 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 2487: MS-OCM Base Medium with 43 mM NaCl and 5 mM sodium acetate

Temperature: 37°C

Atmosphere: 80% H₂, 20% CO₂

Handling Procedures

1. Sterilize the top of the Balch tube by spraying it with 70% ethanol and then flame the top. Filter-sterilize a 2 M formate solution and put in a sealed sterile Balch tube. Exchange air in the head space with 100% nitrogen. The formate can be added to a final concentration of 100 mM to individual tubes of Medium #2487 or to the medium before it is tubed.

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2. If needed, exchange the gas in the test tube for 80% H₂ 20% CO₂.
3. If the medium is pink (see discussion about resazurin) add 0.1 ml Na₂S·9H₂O (1.5% sodium sulfide, stock solution) per 10.0 ml of medium. Let the medium sit at room temperature for 10 to 20 minutes until the resazurin becomes colorless before inoculating.
4. When the Balch tube is ready to inoculate, thaw the frozen vial at room temperature under a gentle stream of oxygen-free gas.
5. For inoculation, use an anaerobic (see "c" below) 1.0 ml syringe tipped with 22-gauge needle, withdraw the cell suspension from the vial and transfer it to the broth. Plate 0.1 ml of the inoculated culture onto a non-selective medium and incubate aerobically at 37°C. Use 0.1 ml of the inoculated culture to inoculate a nonselective aerobic broth and an additional tube of #2487 broth. Incubate the non-selective aerobic broth tubes at 37°C. Incubate the anaerobic tube at 37°C.
6. Growth should be detected in the #2487 broth within 72 to 96 hours. Culture should be feed every 48 hours by replacing the gas in the head space with fresh 80%H₂ - 20% CO₂. There should be no growth detected on the aerobic plate or in the aerobic broth.

ANAEROBIC CONDITIONS:

- a. 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.
- b. 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.
- c. Syringes can be made anaerobic by one of two methods.
 1. Displace the dead space in the syringe with a sterile oxygen-free gas.
 2. Displace the dead space in the syringe with a reducing agent.

Notes

Additional information on this culture is available on the ATCC web site at
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Material Citation

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References

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