

B∆∆-1539[™]

Description

Strain designation: M21

Type strain: No

Storage Conditions

Product format: Frozen

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.

BSL₁

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



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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.

Handling Procedures

- 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 test tube for 80% N₂ 20% CO₂.
- 3. When reducing the media, add 0.1 ml (per 10 ml) of each sterile additions before inoculating. Let the medium sit at room temperature for 1 to 2 hours.
- 4. Allow the frozen vial to thaw under anaerobic conditions. Once thawed, take a gassed 1.0 ml syringe tipped with 22-gauge needle and withdraw the entire contents of the thawed vial and immediately transfer it to a Balch tube.
- 5. Plate 0.1 ml on a non-selective medium to check for aerobic and anaerobic contamination.
- 6. Incubate tubes at 26°C and one plate under an anaerobic atmosphere at 37°C. Incubate non-selective plate aerobically at 37°C to check for purity.
- 7. In 5 days, growth should be evident by the clearing of the broth. No growth should occur on the non-selective plate incubated aerobically or anaerobically.

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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. 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.

Notes

At inoculation, media is brown to orange in color, but after incubation, the media should be clear with black-brown precipitant. The culture has a very low cell density, with only 5 to 6 cells per view under a phase microscope. Cells are rods that are very motile and appear in both singles and pairs. No growth should occur on Tryptic soy agar with 5% Defibernated Sheep blood.

Additional information on this culture is available on the $ATCC^{\otimes}$ web site at <u>www.atcc.org</u>.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Geobacter riflensis (ATCC BAA-1539)*

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References

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

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