Alkaliphilus oremlandii Fisher et al.

BAA-1360™

Description

Strain designation: OhILAs
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.

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 2654: Clostridium OhILA's freshwater Medium
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ATCC Medium 2107: Modified Reinforced Clostridial
Temperature: 18-23°C
Atmosphere: Anaerobic

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 100% N_2 or 80% N_2-20%CO_2.

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. Thaw the frozen vial, and using a gassed 1.0 ml syringe tipped with 22-gauge needle, withdraw the cell suspension from the vial and transfer it to the Balch tube. Plate 0.1 ml of the inoculated culture onto a non-selective medium and incubate the plate aerobically to check for purity. Incubate broth tube at 18-23°C.

ANAEROBIC CONDITIONS:

A. To obtain a fully reduced medium, it is necessary that the 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. 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, titanium citrate and Co-enzyme M (see D).

C. We suggest adding the reducing agent to the medium at least one hour before the medium is to be inoculated. Co-enzyme M (mercaptoethanesulfonic acid) (100 X solution): *Dissolve 5.0 g in 100 ml of deionized water. Distribute into screw cap test tubes, 56 ml per tube and seal with rubber stoppers under N_2 gas. Autoclave to sterilize. Excess tubes can be stored at room temperature for up to 2 months. Co-enzyme M is a compound produced by many methanogens. Some methanogens are sensitive to stronger reducing agents such as sodium sulfide. Co-enzyme M is the standard reducing agent we use when working with methanogens.*

D. Syringes can be made anaerobic by one of two methods.

Notes

In 2-10 days, growth is evident by turbidity. It was noted that the best growth

was obtained when the tubes were incubated on a shaker at 23°C. Cells are rods that occur singly and pairs.

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

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Alkaliphilus oremlandii* Fisher et al. (ATCC BAA-1360)

References

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

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