Thermotoga petrophila Takahata et al.

BAA-488™

Description

Strain designation: JCM 10881 [DSM 13995, RKU-1]
Deposited As: *Thermotoga petrophila* Takahata et al.
Type strain: Yes

Storage Conditions

Product format: Freeze-dried

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 2332: Thermotoga petrophila medium
Temperature: 70°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 80% N_2 -20% CO_2 or 100% N_2.

3. If the medium is pink (see discussion about resazurin) add 1 drop of reducing agent (1.5% sodium sulfide stock solution) for each one or two ml of medium. Let the medium sit at room temperature for 10 to 20 minutes, until the resazurin becomes

colorless, before inoculating.

4. Open the freeze-dried vial according to the enclosed instructions. Take an anaerobic (anaerobic conditions D. below) 1.0 ml syringe tipped with 22-gauge needle and withdraw 0.5 ml of medium from the Balch tube and rehydrate the entire freeze dried pellet. Immediately place the re-hydrated vial under a stream of sterile gas, $80N_2$-$20\%CO_2$ to maintain anaerobicity.

5. Using the same syringe, withdraw the cell suspension from the vial and transfer it to the Balch tube. Inoculate a second pre-reduced tube of medium with 0.5 ml of the rehydrated culture. Plate 0.1 ml of the inoculated culture onto a non-selective medium and incubate aerobically at 37°C. Incubate the broth tube at 70°C.

6. Growth should be detected in the broth within 24 to 48 hours. No growth should be detected on the aerobic plate or broth.

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 oxygen-free gas.

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

Notes

Once growth has been established, the culture should be transferred every 1 or 2 days. The culture will remain viable for 1 week if stored at room temperature and maintained under anaerobic conditions.

When this freeze-dried culture is incubated at elevated temperatures (60°C or above) the cryoprotectant agent precipitates. This precipitation does not interfere with growth of the organism.

Cells appear as short rod that occur singly and in pairs. When examined under phase microscopy the sheath can sometimes be detected.

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: *Thermotoga petrophila* Takahata et al. (ATCC BAA-488)

References

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

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Contact Information

ATCC
10801 University Boulevard
Manassas, VA 20110-2209
USA
US telephone: 800-638-6597
Worldwide telephone: +1-703-365-2700
Email: tech@atcc.org or contact your local distributor