



Product Sheet

Pyrococcus sp. (ATCC®) BAA-2246™)

Please read this **FIRST**



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: *Pyrococcus sp.* (ATCC® BAA-2246™)

Description

Designation: Pikanate 5017

Propagation

Growth Conditions

Temperature: 90-100°C

Atmosphere: Anaerobic; 100% N₂

Propagation Procedure

1. Sterilize the top of the Balch tube (*see below*) by spraying it with 70% ethanol and then flaming the top.
2. If needed exchange the gas in the test tube for 100% N₂. Best results are obtained if the tube is pressurized to approximately 1.52 bar.
3. If the medium is pink (*see discussion about resazurin*) add 2.0 ml of reducing agent (1.5% sodium sulfide, 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. When the Balch tube is ready to inoculate, open the vial according to enclosed instructions.
5. For inoculation, use an anaerobic 1.0 ml syringe (*see below*) tipped with sterile needle. Withdraw 0.5 ml of #2773 broth and use this to rehydrate the freeze-dried pellet. Immediately place the rehydrated vial under a stream of sterile oxygen-free gas.
6. Using the same syringe, transfer the rehydrated cell suspension back to a tube of #2773 broth. Transfer 0.5 ml of the primary tube to a secondary tube of #2773. The cryo-agent used to preserve the culture may form an heavy particulate in the primary tube when incubated at a high temperature. This will make it difficult to detect growth in the primary tube. Plate 0.1 ml of the inoculated culture onto a non-selective medium and incubate aerobically at 37°C. Inoculate a nonselective anaerobic and aerobic broth. Incubate the inoculated tubes at 90 to 100°C.
7. Growth should be detected in the #2773 broth within 24 hours. There should be no growth detected on the aerobic plate. There should be no growth in the nonselective aerobic or anaerobic broth.

ANAEROBIC CONDITIONS:

- a. Balch tube refers to a special type of test tube that is designed to be pressurized and is suited for anaerobic work. The Balch test tubes can be purchased from Bellco glass (www.bellcoglass.com; stock no. 2048-00150).
- 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

References

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

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.

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Disclaimers

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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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