



Pyrococcus horikoshii **Gonzalez et al.**

700860™

Description

Strain designation: JCM 9974 [DSM 12428]

Deposited As: *Pyrococcus horikoshii* Gonzalez 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 1915: *Pyrococcus* medium (DSM medium 377)

Temperature: 95°C

Atmosphere: 100% N₂

Handling Procedures

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 (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. 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 d below*) tipped with 22 gauge needle. Withdraw 0.5 ml of #1915 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 #1915 broth. 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 95°C.

7. Growth should be detected in the #1915 broth within 24 to 48 hours. There should be no growth detected on the aerobic plate, or 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

Notes

D87344, 16S rRNA sequence

- ATCC staff

Cells appear as cocci, singles and in pairs.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Pyrococcus horikoshii* Gonzalez et al. (ATCC 700860)

References

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

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