Prototheca zopfii Kruger

30253™

Description

Strain designation: 48-Y
Deposited As: *Prototheca zopfii* Kruger
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 28: Emmons' modification of Sabouraud's agar/broth
Temperature: 25°C
Culture system: Axenic

Handling Procedures

Culture maintenance:
1. Aseptically transfer a loopful of material to a fresh plate of ATCC medium 28 and spread evenly over the surface with a spread bar.

2. Subculture every 4-6 weeks when incubated at 25C, or every 6-12 months when incubated at 18C.

Cryopreservation:

1. Harvest cells from a culture that is at or near peak density. Add 2-3 ml fresh ATCC medium 28 broth to each plate and wash cells into suspension.

2. Collect cells by centrifugation at 800 x g for 5 min. Adjust the concentration of cells to 2 x 10^6 - 2 x 10^7/ml in fresh medium.

3. While cells are centrifuging prepare a 10% (v/v) solution of sterile methanol in fresh broth medium.

4. Mix the cell preparation and the 10% methanol solution in equal portions. Thus, the final concentration will be 10^6 - 10^7 cells/ml and 5% (v/v) Methanol. The time from mixing of the cell preparation and methanol stock solution to the beginning of the freezing process should be no less than 5 min and no greater than 15 min.

5. Dispense in 0.5 ml aliquots into 1.0 - 2.0 ml sterile plastic screw-capped cryules (special plastic vials for cryopreservation).

6. Place the vials in a controlled rate freezing unit. From room temperature cool at -1°C/min to -40°C. If the freezing unit can compensate for the heat of fusion, maintain rate at -1°C/min through the heat of fusion. At -40°C plunge into liquid nitrogen. Alternatively, place the vials in a Nalgene 1°C freezing apparatus. Place the apparatus at -80°C for 1.5 to 2 hours and then plunge ampules into liquid nitrogen. (The cooling rate in this apparatus is approximately

-1°C/min.)

7. The frozen preparations should be stored in either the vapor or liquid phase of a nitrogen refrigerator. Frozen preparations stored below -130°C are stabile indefinitely. Those stored at temperatures above -130°C are progressively less stabile as the storage temperature is elevated. Vials can be stored between -80 and -70°C for no longer than one week.

8. To establish a culture from the frozen state place an ampule in a water bath set at 35°C. Immerse the vial to a level just above the surface of the frozen material. Do not agitate the vial.

9. Immediately after thawing, do not leave in the water bath, aseptically remove the contents of the ampule and transfer to a test tube containing 5 ml of ATCC medium 28 broth or to the surface of an ATCC medium 28 agar plate.

10. Incubate a test tube culture upright at 25°C with the cap screwed on loosely (loosened one-half turn); incubate a plate upright at 25°C. Subculture every 4-6 weeks when incubated at 25C, or every 6-12 months when incubated at 18C.

Notes

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

While every effort is made to insure authenticity and reliability of strains on deposit, ATCC is not liable for damages arising from the misidentification or misrepresentation of cultures.

ATCC recommends that individuals contemplating commercial use of any culture first contact the originating investigator to negotiate an agreement. Third party distribution of this culture is discouraged, since this practice has resulted in the unintentional spreading of contaminated cultures.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Prototheca zopfii* Kruger (ATCC 30253)

References

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

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