Salpingoeca punica Carr, Richter and Nitsche

50788™

Description

Strain designation: PR1
Deposited As: *Salpingoeca amphoridium* Clark
Type strain: No

Storage Conditions

Product format: Frozen
Storage conditions: -80°C or colder for 1 week, vapor phase of liquid nitrogen for long-term storage

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 802: Sonneborn's Paramecium medium
Instructions for complete medium: ATCC Medium 802 inoculated with *Enterobacter aerogenes* (ATCC® 13048™)
Temperature: 25°C
Culture system: Xenic

Handling Procedures

Storage and Culture Initiation
Frozen ampules packed in dry ice should either be thawed immediately or stored in liquid nitrogen. If liquid nitrogen storage facilities are not available, frozen ampoules may be stored at or below -70°C for approximately one week. **Do not under any**

circumstance store frozen ampules at refrigerator freezer temperatures (generally -20°C). Storage of frozen material at this temperature will result in the death of the culture.

1. One day before thawing the ampule prepare bacterized ATCC medium 802, i.e., inoculate medium with a bacteriological loop of *Enterobacter aerogenes* (ATCC® 13048™) from a nutrient agar slant (ATCC medium 3).
2. To thaw a frozen ampule, place it in a 35°C water bath such that the lip of the ampule remains above the water line. Thawing time is approximately 2 to 3 minutes. Do not agitate the ampule.
3. Immediately after thawing, aseptically transfer the contents to a T-25 tissue culture flask containing 10 ml of bacterized ATCC Medium 802.
4. Screw the cap on tightly and incubate the tube at 25°C.

Culture maintenance:

1. Prepare bacterized ATCC medium 802.
2. Inoculate a T-25 tissue culture flask containing 10.0 mL of bacterized ATCC medium 802 with 0.1 mL from a predominantly encysted *Heteromita globosa* culture.
3. Incubate at 25°C with cap screwed on tightly.
4. For routine maintenance subculture every 10-14 d.

Cryopreservation:

1. Harvest cells from a culture that is at or near peak density by centrifugation at 800 x g for 5 min.
2. Adjust the concentration of cells to 2 x 10^6 - 10^7/mL in fresh medium.
3. While cells are centrifuging prepare a 20% (v/v) solution of sterile DMSO in fresh medium.
    a. Add 2.0 mL of DMSO to an ice cold 20 x 150 mm screw-capped test tube;
    b. Place the tube on ice and allow the DMSO to solidify (~5 min) and then add 8.0 mL of ice cold medium;
    c. Invert several times to dissolve the DMSO;
    d. Allow to warm to room temperature.
4. Mix the cell preparation and the DMSO in equal portions. Thus, the final concentration will be 10^6 - 10^7 and 10% (v/v) DMSO. The time from the mixing of the cell preparation and DMSO stock solution before the freezing process is begun should be no less than 15 min and no longer than 30 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. If frozen material is to be stored at temperatures between -130°C and -70°C the shelf life should be empirically tested, i.e., remove stored material at intervals to determine die-off rate.
8. To establish a culture from the frozen state place an ampule in a water bath set at 35°C. Immerse the ampule to a level just above the surface of the frozen material. Do not agitate the ampule.
9. Immediately after thawing, do not leave in the water bath, aseptically remove the contents of the ampule and inoculate a T-25 tissue culture flask containing 10 mL of bacterized ATCC medium 802.
10. Incubate with the cap screwed on tightly at 25°C.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Salpingoeca punica* Carr, Richter and Nitsche (ATCC 50788)

References

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

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Revision

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