Stephanopogon apogon Borror

50096™

Description

Strain designation: BCP
Deposited As: *Stephanopogon apogon* Borror
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 1525: Seawater Sonneborn's Paramecium Medium
ATCC Medium 1405: HESNW medium
ATCC Medium 1361: Marine flagellate medium
Instructions for complete medium:

Media: ATCC® Medium 1525 and one of the alternate media listed below, combined in equal parts and inoculated with *Klebsiella pneumoniae* subsp. *pneumoniae* (ATCC® 700831™) or *Enterobacter aerogenes* (ATCC® 13048™)

Alternate media: ATCC® Medium 1405 HESNW medium, ATCC® Medium 1361 Marine Flagellate medium (both media augmented with two sterile rice grains added to culture flask)

Note about growth media: ATCC Media 1405 and 1361 will each support moderate

growth of the prey organism when used on their own, but will support more rapid and dense growth when combined in an equal-parts mixture with previously-bacterized ATCC Medium 1525.

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 ampules 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. 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. Do not leave ampule in water bath after it is thawed. Do not allow ampule to overheat.
2. Add the thawed contents to a T-25 flask containing 10 ml of an equal-parts mixture of ATCC medium 1525 and either ATCC medium 1405 or ATCC medium 1361, bacterized with *Klebsiella pneumoniae* subsp. *pneumoniae* (ATCC® 700831™) or *Enterobacter aerogenes* (ATCC® 13048™).
3. Aseptically transfer 1-2 ml from a thriving culture of *Rhynchomonas* to the T-25 flask. Incubate the culture at 25°C with the cap screwed on tightly.

Culture maintenance: Subculture every 7-14 days to a fresh T-25 flask of the prey organism in bacterized medium as follows:

1. Maintain growing cultures of *Rhynchomonas* separately in T-25 tissue culture flasks containing 10 ml of an equal-parts mixture of ATCC medium 1525 and either ATCC medium 1405 or ATCC medium 1361, bacterized with *Klebsiella pneumoniae* subsp. *pneumoniae* (ATCC® 700831) or *Enterobacter aerogenes* (ATCC® 13048).
2. When the prey organism is at or near peak density, rub the bottom surface of

an encysted culture of *Stephanopogon* with a cell scraper to detach the cysts, vigorously agitate, and aseptically transfer a 0.5 ml aliquot of the cyst suspension to the prey culture. Incubate at 25°C with the cap screwed on tightly.

Reagents for cryopreservation:

Cryoprotective Solution
DMSO, 2.0 ml
Fresh growth medium w/o bacteria, 8.0 ml

Cryopreservation:

1. Mix the components in the order listed. When the medium is added to the DMSO the solution will warm up due to chemical heat.
2. Harvest *Stephanopogon* cells or cysts from a culture that has recently passed peak density by filtration and centrifugation at 800 x g for 5 min.
3. Adjust the concentration of cysts at least 2 x 10^5/ml in fresh medium.
4. Mix the cell preparation and the cryoprotective solution in equal portions.
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 vials in a controlled rate freezing unit. From room temperature cool at -1°C/min to -40°C. If freezing unit can compensate for the heat of fusion, maintain rate at -1 C/min through heat of fusion. At -40°C plunge ampules 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. Ampules are stored in either the vapor or liquid phase of a nitrogen refrigerator.
8. To establish a culture from the frozen state place the vial in a 35°C water bath. Immerse the vial to a level just above the surface of the frozen material. Do not agitate the vial. Immediately after thawing, do not leave in water bath, aseptically remove the contents of the ampule and inoculate into a T-25 tissue culture flask containing 10 ml of an equal-parts mixture of ATCC medium 1525 and either ATCC medium 1405 or ATCC medium 1361, bacterized with *Klebsiella pneumoniae* subsp. *pneumoniae* (ATCC® 700831?) or *Enterobacter aerogenes* (ATCC® 13048™).
9. Aseptically transfer 1-2 ml from a thriving culture of *Rhynchomonas* to the T-25 flask. Incubate the culture at 25°C with the cap screwed on tightly.

Once the culture is established, follow the protocol for maintenance of culture.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Stephanopogon apogon* Borror (ATCC 50096)

References

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

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