



Product Sheet

Sphaerothecum destruens (ATCC® 50615™)

Please read this **FIRST**

Storage Temp.
Frozen Cultures:
-70°C for 1 week;
liquid N₂ vapor
for long term
storage



Freeze-dried Cultures:
2-8°C

Live Cultures:
See Protocols
section for
handling
information



Biosafety Level
2

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: *Sphaerothecum destruens* (ATCC® 50615™)

American Type Culture Collection
PO Box 1549
Manassas, VA 20108 USA
www.atcc.org

800.638.6597 or 703.365.2700
Fax: 703.365.2750
Email: Tech@atcc.org

Or contact your local distributor



Description

Strain Designation: BBCA
Deposited Name: Rosette agent
Depositor: K Arkush
Isolation: Kidney tissue of winter-run Chinook salmon, Bodega Bay, CA, 1994



Notes

CHSE-214 cells (ATCC® CRL-1681™) are no longer available for domestic or international distribution due to being listed as a threatened species on the endangered species list. ASK (ATCC® CRL-2747™) is the only other salmonid cell line available at ATCC, but its capacity to support the growth of *Sphaerothecum destruens* has not been tested at ATCC.



Propagation

Growth Conditions
Temperature: 15°C
Atmosphere: Microaerophilic
Culture System: CHSE-214 cells (ATCC® CRL-1681™)

Instructions for Complete Medium

Eagle's Minimum Essential Medium (EMEM) (ATCC® cat. 30-2003™) with 2 mM L-glutamine and Earle's BSS adjusted to contain 1.5 g/L sodium bicarbonate, 0.1 mM non-essential amino acids, and 1.0 mM sodium pyruvate; supplemented with 7.5% fetal bovine serum (ATCC® cat. 30-2020™).
Cultured on CHSE-214 cell line (ATCC® CRL-1681™).



Protocols

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 ampoules 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.
2. Immediately after thawing, aseptically transfer contents to a T-25 tissue culture flask containing a fresh monolayer of cells (ATCC® CRL-1681™) and 10 mL ATCC® 30-2003™ with 7.5% (v/v) HIFBS.
3. Outgas the flask for 10 seconds with a 95% air, 5% CO₂ gas mixture.
4. Incubate in a 15°C CO₂ incubator with the cap screwed on tightly.

Culture Maintenance

Cell Line Maintenance:

1. To establish a cell culture from the frozen state place an ampule in a water bath set at 35°C (2-3 min). Immerse the vial just sufficiently to cover the frozen material. Do not agitate the vial.
2. Immediately after thawing, aseptically remove the contents of the ampule and inoculate into 10.0 mL of fresh ATCC® 30-2003™ with 10% (v/v) Heat-Inactivated Fetal Bovine Serum (HIFBS)* in a T-25 tissue culture flask.
3. Outgas the flask for 10 seconds with a 95% air, 5% CO₂ gas mixture.
4. Incubate in a 20-22°C CO₂ incubator with the cap screwed on tightly.
5. Change the medium 1-2 times per week.

*Fetal bovine serum is available from ATCC (catalog number 30-2020™). Serum is heat-inactivated by exposure to 56°C for 30 minutes. This treatment will inactivate proteins of the complement pathway. Remove the serum from the refrigerator and aseptically distribute in 100 mL aliquots to sterile 125 mL screw-capped bottles. Immerse bottles in a 35°C water bath for 5 minutes. Do not directly transfer bottles from the refrigerator to 56°C. Transfer the bottles to a 56°C water bath and begin timing for 30 minutes. To avoid contamination, do not allow the level of the water in the bath to come in contact with the lip of the screw cap. It is best to leave one inch between the serum level in the bottle and the lip of the cap and to fill the water bath to a level just slightly above the level of the serum. To assure even heating of the serum, swirl the bottle(s) every ten minutes.



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Note: Some suppliers provide serum already heat-inactivated.

Transferring the Cell Line:

1. When the cell line forms a confluent layer, remove all the medium and replace it with 3 mL of Phosphate Buffered Saline (PBS) (ATCC® cat. 30-2200™). Incubate T-25 flask at 35-37°C for 25-30 min.
2. Remove all the PBS and replace it with 2 mL of 0.25% (w/v) trypsin dissolved in Hank's Balanced Salt Solution (ATCC® cat. 30-2101™).
3. Gently distribute the trypsin over the monolayer, remove the trypsin, and place the flask at 20-22°C for 10 min.
4. Add 2 mL of ATCC® 30-2003™ with 10% (v/v) HIFBS and detach any cells still adherent by alternately aspirating the medium into a pipette and discharging the contents over the monolayer.
5. Distribute the cell suspension in 0.5 mL aliquots to 4 T-25 flasks containing 10 mL fresh ATCC® 30-2003™ with 10% (v/v) HIFBS.
6. Outgas the flask for 10 seconds with a 95% air, 5% CO₂ gas mixture.
7. Incubate in a 20-22°C CO₂ incubator with the cap screwed on tightly.

Maintenance of *Sphaerothecum* Culture:

1. Remove the medium from a fresh confluent monolayer of cells (ATCC® CRL-1681™) in a T-25 tissue culture flask and replace it with 10 mL of ATCC® 30-2003™ with 7.5% (v/v) HIFBS.
2. To transfer the *Sphaerothecum* culture, remove the old medium containing the organism and centrifuge at 1300 x g for 10 min.
3. Remove all but 0.5 mL of the supernatant and resuspend the cell pellet. Transfer the resuspended pellet to the fresh flask of cells (ATCC® CRL-1681™).
4. Outgas the flask for 10 seconds with a 95% air, 5% CO₂ gas mixture.
5. Incubate in a 15°C CO₂ incubator with the cap screwed on tightly.



Cryopreservation

Reagents

Cryoprotective Solution

DMSO 2.0 mL

Fresh growth medium (7.5% serum) 8.0 mL

Harvest and Preservation

1. To harvest the *Sphaerothecum* culture, detach any remaining tissue culture cells (infected and uninfected) by scraping the surface of the flask with a cell scraper.
2. Transfer the cell suspension (including parasites) to 15 mL plastic centrifuge tubes. Centrifuge at 1000 x g for 10 min.
3. Remove all but 0.5 mL of the supernatant from each tube, resuspend the cell pellets, and pool them to a single tube.
4. Optionally pass the resulting cell suspension through a syringe equipped with a 27 gauge 1/2 in needle to break up any remaining cells.
5. Adjust the parasite concentration to 2.0 - 4.0 x 10⁷ cells/mL with fresh medium or PBS.
NOTE: If the concentration of parasites is too low, centrifuge at 1000 x g for 10 min and resuspend in the volume of fresh medium or PBS required to yield the desired concentration.
6. Prepare a cryoprotective solution containing 20% (v/v) DMSO and 7.5% (v/v) HIFBS in fresh medium or PBS.
7. Mix the cell preparation and cryoprotective solution in equal portions. The final concentration will be 1.0 - 2.0 x 10⁷ cells/mL, 10% DMSO, and 7.5% HIFBS. The time from the mixing of the cell preparation and cryoprotective solution to the start of the freezing process should be no less than 15 min. and no more than 30 min.
NOTE: To prevent culture contamination, penicillin-streptomycin solution (ATCC® 30-2300™) may be added to a final concentration of 50 to 100 I.U./mL penicillin and 50 to 100 µg/mL streptomycin.
8. Dispense in 0.5 mL aliquots to 1.0-2.0 mL sterile plastic screw-capped cryovials (special plastic vials for cryopreservation).
9. 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.)
10. Store frozen ampules in either the vapor or liquid phase of a nitrogen refrigerator.
11. 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



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ampule in water bath after thawed.

12. Immediately after thawing, aseptically transfer contents to a T-25 tissue culture flask containing a fresh monolayer of cells (ATCC® CRL-1681™) and 10 mL ATCC® 30-2003™ with 7.5% (v/v) HIFBS.
13. Outgas the flask for 10 seconds with a 95% air, 5% CO₂ gas mixture.
14. Incubate in a 15°C CO₂ incubator with the cap screwed on tightly.
15. Once the culture is established, follow the protocol for maintenance of culture.



References

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



Biosafety Level: 2

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.

ATCC Warranty

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Disclaimers

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

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