



Product Sheet

Blepharisma stoltei (ATCC® 30299™)

Please read this **FIRST**



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: *Blepharisma stoltei* (ATCC® 30299™)

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: Federsee
Deposited Name: *Blepharisma stoltei* Isquith
Depositor: AJ Repak
Isolation:
Lake Federsee, Federsee, Germany, 1963

Propagation

Growth Conditions
Temperature: 25.0°C

Instructions for Complete Medium

ATCC Medium 802 inoculated with *Klebsiella pneumoniae* subsp. *pneumoniae* (ATCC® 700831).

Culture Maintenance

Subculture every two weeks to a fresh T-25 flask of bacterized medium in the following manner:

1. Vigorously agitate the flask and aseptically transfer 0.5 ml from a growing culture to a T-25 tissue culture flask containing 10.0 ml of ATCC medium 802 bacterized with *Klebsiella pneumoniae* subsp. *pneumoniae* (ATCC® 700831)
2. Incubate flask at 25°C with the cap loosened one half turn.

Cryopreservation

Cryoprotective Solution

DMSO	2.0 ml
Fresh growth medium w/o bacteria	8.0 ml

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 cells from a culture that is at or near peak density by filtration and centrifugation at 500 x g for 5 min.
3. Adjust the concentration of cells at least 2×10^6 /ml in fresh medium.
4. Allow the concentrated cells to remain undisturbed for approximately 1 hour. Make sure that the concentrated suspension has plenty of air, i.e., the surface to volume ratio of the suspension should be high. This resting period allows the cells to repair membrane damage that occurs during centrifugation.
5. Mix the cell preparation and the cryoprotective solution in equal portions.
6. Dispense in 0.5 ml aliquots into 1.0 - 2.0 ml sterile plastic screw-capped cryules (special plastic vials for cryopreservation).
7. 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.)
8. Ampules are stored in either the vapor or liquid phase of a nitrogen refrigerator.
9. 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 ATCC medium 802 bacterized with *Klebsiella pneumoniae* subsp. *pneumoniae* (ATCC® 700831).
10. Incubate at 25°C with the cap loosened one half turn.
11. Once the culture is established, vigorously agitate the flask and aseptically transfer 0.5 ml to 10.0 ml of bacterized ATCC medium 802.
12. Follow the protocol for maintenance of culture.

References

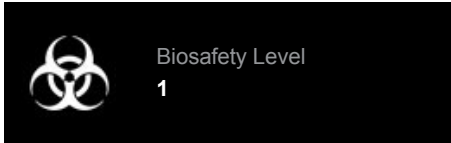
References and other information relating to this product are available online at www.atcc.org.



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Biosafety Level: 1

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

This product is intended for laboratory research purposes only. It is not intended for use in humans.

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

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