



# ***Blepharisma stoltei*** **Isquith**

**30299™**

## **Description**

**Strain designation:** Federsee

**Deposited As:** *Blepharisma stoltei* Isquith

**Type strain:** No

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## **Storage Conditions**

**Product format:** Test tube

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## **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.

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## **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.

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## Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at [www.atcc.org](http://www.atcc.org).

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## Growth Conditions

### Medium:

ATCC Medium 802: Sonneborn's Paramecium medium

**Instructions for complete medium:** ATCC Medium 802 inoculated with *Klebsiella pneumoniae* subsp. *pneumoniae* (ATCC® 700831)

**Temperature:** 25°C

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## Handling Procedures

### 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.

**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 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  $2 \times 10^6$ /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.

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## Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Blepharisma stoltei* Isquith (ATCC 30299)

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## References

References and other information relating to this material are available at [www.atcc.org](http://www.atcc.org).

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