**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**
1

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**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: *Willaertia magna* (ATCC® 50035™)

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**Description**

**Strain Designation:** Z503  
**Deposited Name:** *Willaertia magna* De Jonckheere et al.  
**Depositor:** JF De Jonckheere  
**Isolation:** Bovine feces, France

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**Propagation**

**Growth Conditions**
- Temperature: 35°C
- Culture System: Axenic

**Medium**
- ATCC® Medium 1034: Modified PYNFH medium (Available from ATCC as ATCC cat. no. 327-X)

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**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 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 until thawed (2-3 min). Immerse the ampule enough to cover only the frozen material. Do not agitate the ampule.
2. Immediately after thawing, aseptically transfer contents to either a plastic screw-capped tube containing 5 mL ATCC medium 1034, or to a T-25 tissue culture flask containing 10 mL ATCC medium 1034. Incubate at 35°C with the cap screwed on tightly (incubate a test tube on a 15° horizontal slant).

**Culture Maintenance**

1. Vigorously agitate a culture at or near peak density and aseptically transfer 0.1-0.2 mL to a fresh tube of ATCC medium 1034.
2. Incubate at 35°C with the cap screwed on tightly (incubate a test tube on a 15° horizontal slant).

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**Cryopreservation**

**Harvest and Preservation**

1. To achieve the best results set up cultures with several different inocula (e.g. 0.25 mL, 0.5 mL, 1.0 mL). Harvest cell cultures and pool when the lowest inoculum is at or near peak density.
2. If the cell concentration exceeds the required level do not centrifuge, but adjust the concentration to between 2 x 10⁶ and 2 x 10⁷ cells/mL with fresh medium. If the concentration is too low, centrifuge at 600 x g for 5 min and resuspend the pellet in the volume of fresh medium required to yield the desired concentration.
3. While cells are centrifuging prepare a 15% (v/v) solution of sterile DMSO as follows: Add the required volume of DMSO to a glass screw-capped test tube and place it in an ice bath. Allow the DMSO to solidify. Add the required volume of refrigerated medium. Dissolve the DMSO by inverting the tube several times. *NOTE: If the DMSO solution is not prepared on ice, an exothermic reaction will occur that may precipitate certain components of the medium.
4. Mix the cell preparation and the DMSO in equal portions. Thus, the final concentration will be between 10⁶ and 10⁷ cells/mL and 7.5% (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 60 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 are stored in either the vapor or liquid phase of a nitrogen freezer.
8. To establish a culture from the frozen state place an ampule in a water bath set at 35°C (2-3 min). Immerse the vial just sufficient to cover the frozen material. Do not agitate the vial.

9. Immediately after thawing, aseptically remove the contents of the ampule and inoculate into 10 mL of fresh ATCC medium 1034 in a T-25 tissue culture flask. Incubate at 35°C.

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References

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

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ATCC Warranty

The viability of ATCC® products is warranted for 30 days from the date of shipment, and is valid only if the product is stored and cultured according to the information included on this product information sheet. ATCC lists the media formulation that has been found to be effective for this strain. While other, unspecified media may also produce satisfactory results, a change in media or the absence of an additive from the ATCC recommended media may affect recovery, growth and/or function of this strain. If an alternative medium formulation is used, the ATCC warranty for viability is no longer valid.

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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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Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at [www.atcc.org](http://www.atcc.org).

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Additional information on this culture is available on the ATCC web site at [www.atcc.org](http://www.atcc.org).

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