**Spiroplasma sp. (ATCC® 29761™)**

**Description**

**Designation:** 277F  
**Deposited Name:** Spiroplasma citri Saglio et al.

**Propagation**

**Medium**  
ATCC® Medium 2611: Spiroplasma medium

**Growth Conditions**  
**Temperature:** 30.0°C  
**Atmosphere:** 5% CO₂

**Propagation Procedure**

1. Follow instructions as suggested for the culturing of Mollicutes:  
   **PROCEDURES FOR PROPAGATING MOLLICUTES:**
   a) Open the vial according to the enclosed instructions.  
   b) Using a Pasteur or 1.0 ml pipette, withdraw approximately 0.5 to 1.0 ml from a tube containing 5.0 ml. Rehydrate the pellet.  
   c) Aseptically transfer this aliquot back into the tube. Mix well.  
   d) Make serial dilutions by transferring 0.5 ml from the original tube to a tube containing 4.5 ml. Repeat process by transferring 0.5 ml from the second to a third tube, etc. Dilutions are important, not only for titration purposes, but also to keep culture in varying stages of growth. Many strains will die out rapidly once acid or alkaline conditions are reached. It is recommended to prepare several dilutions from the initial tube as the cryoprotectant used in the freeze-drying process often inhibits growth.  
   e) Use an uninoculated tube of broth to serve as a control.  
   f) Plates may be inoculated to check colonial morphology. You can also spot each dilution on the surface of plate (4 or more/plate) to determine the number of colony-forming units. However, not all strains do well on solid medium.  
   g) Incubate all tubes and plates under the recommended conditions and appropriate temperature. The time necessary for growth will vary from strain to strain. Growth on plates generally requires additional incubation.  
   h) Depending on the medium used, growth will be indicated by increased turbidity, a color change, or both.  
2. For long term storage of Spiroplasma species, freeze-drying or freezing is recommended. Liquid nitrogen storage is the best method. Optimally grown cells are centrifuged at 9000 rpm for 30 minutes, the supernatant poured off, and the packed cells resuspended in a smaller amount of the recommended broth. To this, add an equal amount of sterile 20% glycerol as a cryoprotectant. This suspension is aliquoted into small plastic vials and stored at -70°C or below.

**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: *Spiroplasma sp.* (ATCC® 29761™)
Please read this FIRST

**Biosafety Level:** 2

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**Notes**

Store vials at freezer temperatures until ready to use.

**References**

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

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