**Mycoplasma pneumoniae**

(ATCC® 15531-TTR™)

**Please read this FIRST**

**Storage Temp.**

Frozen: -80°C or colder

Freeze-Dried: 2°C to 8°C

**Live Culture: See Propagation Section**

**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: Mycoplasma pneumoniae (ATCC® 15531-TTR™)

**Description**

**Designation:** FH strain of Eaton Agent [NCTC 10119]

**Deposited Name:** Mycoplasma pneumoniae

**Product Description:** Type strain. This organism is the first Mycoplasma known to be the etiological agent of a human disease.

**Propagation**

**Medium**

ATCC® Medium 2611: Spiroplasma medium

**Growth Conditions**

**Temperature:** 37°C

**Atmosphere:** Broth: Aerobic; Plates: 5% CO₂ or in a candle jar

**Propagation Procedure**

1. Follow instructions as suggested for the culturing of Mollicutes:

**PROCEDURES FOR PROPAGATING MOLLICUTES:**

   a. Allow the vial to thaw on bench top.
   b. Using a Pasteur or 1.0 mL pipette, withdraw entire contents of vial.
   c. Aseptically transfer this aliquot back into the maintenance media 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. This strain starts to show turbidity in the first few dilution tubes within 48 to 72 hours. Additional incubation may be required for growth on solid medium.

3. Subsequent, fresh transfers grow in 24-48 hours. The freeze drying process and the cryoprotectant occasionally slows the growth rate of the initial culture.

**Notes**

Store vials at freezer temperatures until ready to use.

ATCC Medium 988 Spiroplasma medium SP-4 can be used as an alternate.

Using a candle jar for CO₂ conditions may be used for those strains whose medium has an indicator present.

CO₂ incubators may lower the pH of the medium enough to cause a color change. This change may make it difficult to observe growth with those strains that show little turbidity.

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

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

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