



Product Sheet

Mycoplasma pneumoniae (ATCC® 15531™)

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™)

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

Designation: FH strain of Eaton Agent [NCTC 10119]

Deposited Name: *Mycoplasma pneumoniae* Somerson et al.

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

Propagation

Medium

ATCC® Medium 988: Spiroplasma medium SP-4

ATCC® Medium 2611: *Spiroplasma* medium

Growth Conditions

Temperature: 37°C

Atmosphere: Broth: Aerobic; Agar: 5% CO₂ or candle jar

Propagation Procedure

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

PROCEDURES FOR PROPAGATING *MOLLICUTES*:

- a. Add an additional 5% heat-inactivated fetal bovine serum to medium #988. 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 T-flask containing 2.5 mL of the recommended broth. Rehydrate the entire pellet.
 - c. Aseptically transfer this aliquot back into the tube. Mix well.
 - d. Make serial dilutions by transferring 0.25 mL from the original tube to a tube containing 2.5 mL. Repeat process by transferring 0.25 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 colony morphology. You can also spot each dilution on the surface of plate (4 or more/plate) to determine the number of colony-forming units. Growth on agar may take up to 2 weeks when grown at 37°C in 5% CO₂.
2. *Mycoplasma pneumoniae* strains are very slow growing and produce a very light turbidity. Growth in broth is best observed after 10 to 14 days of incubation. It usually takes at least seven days for the first T-flasks to start showing growth. Growth is easily recognized by an indicator change from red to orange to yellow. The cells are best transferred when the medium is orange. After medium changes to yellow, cells have started to die.
 3. For long term storage of *Mycoplasma pneumoniae*, 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 #2611 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 colder.

Notes

Growth may take up to 2 weeks.

This strain requires an additional 5% heat-inactivated fetal bovine serum be added to ATCC Medium #988 to sustain growth of this strain.

The flasks should be checked to make sure media is not evaporating and to add media as needed to maintain the volume.

Store vials at freezer temperatures until ready to use.

We have found that using a *candle jar* for CO₂ conditions works better 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.

Purified genomic DNA of this strain is available as ATCC® 15531D™.

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.



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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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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

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

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