Mycoplasmoides pneumoniae (Somerson et al.) Gupta et al.

15531™

Description

Mycoplasmoides pneumoniae FH strain of Eaton Agent [NCTC 10119] is a whole genome-sequenced type strain strain and the first *Mycoplasma* known to be the etiological agent of a human disease. This strain was isolated by Hayflick from monkey kidney tissue-culture fluids of the FH strain (Eaton Agent Virus) supplied by C. Liu, who recovered this strain in embryonated eggs from a student with atypical pneumonia. This can be used in media testing, respiratory research, and pharmaceutical and personal care.

Strain designation: FH strain of Eaton Agent [NCTC 10119]
Deposited As: *Mycoplasma pneumoniae* Somerson et al.
Type strain: Yes

Storage Conditions

Product format: Freeze-dried
Storage conditions: 2°C to 8°C

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.

BSL 2

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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.

Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at www.atcc.org.

Growth Conditions

Medium:
ATCC Medium 0988: Spiroplasma medium SP-4
Temperature: 37°C
Atmosphere: Broth: Aerobic; Plates: 5% CO_2

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Handling Procedures

1. Follow instructions as suggested for the culturing of Mollicutes

PROCEDURES FOR PROPAGATING MOLLICUTES

a. Open the vial.
b. Using a Pasteur or 1.0 mL pipette, withdraw approximately 0.5 to 1.0 mL from a tube or T-flask containing 2.5 mL. Rehydrate the 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 or T-flask to a tube or T-flask containing 2.25 mL. Repeat process by transferring 0.25 mL from the second to a third tube or T-flask, 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 or T-flask 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 or T-flasks 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. Tubes or T-flasks are incubated aerobically; plates are incubated under 5% CO_2. Using higher concentrations of CO_2 will result in lowering the pH of the medium. The incubation temperature is 37°C.
3. *M. pneumoniae* strains are very slow growing and produce a very light turbidity. This strain usually takes at least seven to 14 days for the first tubes or 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. Subsequent transfers should grow in a shorter span of time.

4. For long term storage of *M. 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 #988 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.

Notes

Commercially available SP4 Glucose (Remel catalog # R112585 or #R20376 for broth, and Remel catalog # R20276 for agar) may yield the best growth.

This strain requires an additional 5% heat-inactivated fetal bovine serum be added to ATCC Medium #988 or #2611 or SP4 Glucose broths 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.

Purified genomic DNA of this strain is available (ATCC 15531D).

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

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Mycoplasmoides pneumoniae* (Somerson et al.) Gupta et al. (ATCC 15531)

References

References and other information relating to this material are available at www.atcc.org.

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Revision

This information on this document was last updated on 2025-10-16

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