ATCC

Product Sheet

Mycoplasma hyopneumoniae (ATCC® 25934™)

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 hyopneumoniae* (ATCC® 25934™)

American Type Culture Collection
PO Box 1549
Manassas, VA 20108 USA
www.atcc.org

800.638.6597 or 703.365.2700
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Or contact your local distributor

Description

Designation: [NCTC 10110; J]
Deposited Name: *Mycoplasma suipneumoniae* Goodwin et al.
Product Description: Type strain. Referred to as the type strain of *Mycoplasma suipneumoniae*. Genome sequenced strain.

Propagation

Medium
ATCC® Medium 1699: Revised Mycoplasma medium

Growth Conditions
Temperature: 37°C
Atmosphere: Broth: Aerobic; Plates: 5% CO_2

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 single tube (5 to 6 mL) of Medium #1699. 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 2.25 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 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 is indicated by increased turbidity, a color change, or both.
   i. Tubes are incubated aerobically, plates are incubated under 5% CO_2 or in a candle jar. The incubation temperature is 37°C.
2. *Mycoplasma hyopneumoniae* strains are very slow growing and produce a flocculent turbidity. It may be necessary to hold tubes up to a light source to detect growth. Initial growth may take up to a week to be detected in first tubes. An indicator change from red to orange to yellow is very slow. The best indicator of growth will be the flocs in the broth medium. **The cells are best transferred when the medium is orange. After medium changes to yellow, cells have started to die.** Subsequent transfers usually grow in 48+ hours, but may take longer depending on size of inoculum.
3. After broth growth is established, freshly inoculated plates will take three or more days to produce colonies. The colonies vary in size from tiny to small; rough with irregular margin. They do not exhibit the usual "fried egg" appearance.
4. For long term storage of *Mycoplasma hyopneumoniae,* 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 #1699 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

Store vials at 4°C until ready to use.

We have found that using a candle jar for CO_2 conditions works better for those strains whose medium has an indicator present. CO_2 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® 25934D™.

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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.

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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Disclaimers

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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

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