



***Mycoplasma hyopneumoniae* Mare and Switzer**

25095™

Description

Strain designation: [VMRI 11]

Deposited As: *Mycoplasma hyopneumoniae* Mare and Switzer

Type strain: No

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

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 1699: Revised Mycoplasma medium

Temperature: 37°C

Atmosphere: Broth: Aerobic; Plates: 5% CO₂

Handling Procedures

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 0.25 mL from a tube of #1699 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 first tube to a second 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 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. 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% CO₂ 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

This strain does not grow well on agar. Growth is visible in broth before the indicator changes. Growth will take from 7 to 12 days.

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.

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: *Mycoplasma hyopneumoniae* Mare and Switzer (ATCC 25095)

References

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

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