Dileptus americanus Kahl

50259™

Description

Strain designation: RV
Deposited As: *Dileptus americanus* Kahl
Type strain: No

Storage Conditions

Product format: Frozen
Storage conditions: -80°C or colder for 1 week, vapor phase of liquid nitrogen for long-term storage

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 1

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 802: Sonneborn's Paramecium medium
ATCC Medium 1323: Page's balanced salt solution (PBS)
ATCC Medium 5080: Dryl's Solution
Instructions for complete medium: ATCC® Medium 802 inoculated with *Klebsiella pneumoniae* subsp. *pneumoniae* (ATCC® 700831™) or *Enterobacter aerogenes* (ATCC® 13048™). ATCC Medium 802 will support growth of b
Temperature: 20-25°C

Handling Procedures

Storage and Culture Initiation
Frozen ampules packed in dry ice should either be thawed immediately or stored in liquid nitrogen. If liquid nitrogen storage facilities are not available, frozen ampoules

may be stored at or below -70°C for approximately one week. **Do not under any circumstance store frozen ampules at refrigerator freezer temperatures (generally -20°C).** Storage of frozen material at this temperature will result in the death of the culture.

1. To thaw a frozen ampule, place it in a 35°C water bath such that the lip of the ampule remains above the water line. Thawing time is approximately 2 to 3 minutes. Do not agitate the ampule. Do not leave ampule in water bath after it is thawed. Do not allow ampule to overheat.
2. Add the thawed contents to a T-25 flask containing 10 mL of ATCC medium 802 bacterized with *Klebsiella pneumoniae* subsp. *pneumoniae* (ATCC® 700831™) or *Enterobacter aerogenes* (ATCC® 13048™).
3. Aseptically transfer 1-2 mL of washed *Tetrahymena* to the T-25 flask (see section on CULTURE MAINTENANCE below). Incubate the culture at 20-25°C.

Culture maintenance:

Periodically add prey organisms as follows:

1. Maintain growing cultures of *Tetrahymena* separately at 20-25°C in T-25 tissue culture flasks containing 10 mL ATCC medium 1034 without serum.
2. Prepare washed *Tetrahymena* as follows: Remove 5-10 mL from a culture at or near peak density, centrifuge at 300 x g for 3 min, quickly remove most of the supernatant (leaving approx. 1 mL), then resuspend cells in 10 mL ATCC medium 1323 or similar. Centrifuge and resuspend cells again as above. Repeat this washing step at least twice.
3. When the *Dileptus* have consumed all prey *Tetrahymena*, add 0.5-1.0 mL of washed *Tetrahymena* prepared in step 2. The feeding interval will depend on the number of predators present and the culture density of the washed prey.
4. The *Dileptus* may be passaged to a T-25 flask by gently rubbing the flask surface with a spread bar to dislodge any attached cells or cysts, then transferring 0.5 to 2 mL to a fresh T-25 flask containing 10 mL ATCC medium 802 bacterized with *Klebsiella pneumoniae* subsp. *pneumoniae* (ATCC® 700831™) or *Enterobacter aerogenes* (ATCC® 13048™). Incubate the culture at 20-25°C, feeding periodically with washed *Tetrahymena* as described above.

Reagents for cryopreservation: Cryoprotective Solution
DMSO, 1.5 mL
Fresh growth medium w/o bacteria, 8.5 mL

Cryopreservation:

Harvest and Preservation

1. Mix the components in the order listed. When the medium is added to the DMSO the solution will warm up due to chemical heat.
2. Harvest *Dileptus* cells or cysts from a culture that has recently passed peak density by filtration and centrifugation at 200-300 x g for 5 min.
3. Adjust the concentration of cells or cysts at least 2 x 10^4/mL in fresh medium.
4. Mix the cell preparation and the cryoprotective solution in equal portions.
5. Dispense in 0.5 mL aliquots into 1.0 - 2.0 mL sterile plastic screw-capped cryules (special plastic vials for cryopreservation).
6. Place vials in a controlled rate freezing unit. From room temperature cool at -1°C/min to -40°C. If freezing unit can compensate for the heat of fusion, maintain rate at -1°C/min through heat of fusion. At -40°C plunge ampules into liquid nitrogen. Alternatively, place the vials in a Nalgene 1°C freezing apparatus. Place the apparatus at -80°C for 1.5 to 2 hours and then plunge ampules into liquid nitrogen. (The cooling rate in this apparatus is approximately -1°C/min.)
7. Ampules are stored in either the vapor or liquid phase of a nitrogen refrigerator.
8. To establish a culture from the frozen state place the vial in a 35°C water bath. Immerse the vial to a level just above the surface of the frozen material. Do not agitate the vial. Immediately after thawing, do not leave in water bath, aseptically remove the contents of the ampule and inoculate into a T-25 tissue culture flask containing 10 mL of ATCC medium 802 bacterized with *Klebsiella pneumoniae* subsp. *pneumoniae* (ATCC® 700831™) or *Enterobacter aerogenes* (ATCC® 13048™).
9. Aseptically transfer 1-2 mL of washed *Tetrahymena* to the T-25 flask (see section on CULTURE MAINTENANCE). Incubate the culture at 20-25°C.
   Once the culture is established, follow the protocol for maintenance of culture.

Notes

This strain must be fed with live *Tetrahymena* (i.e., ATCC® 30005™ or similar, not provided). The *Tetrahymena* should be maintained separately and fed to *Dileptus* at regular intervals. *Dileptus* will eventually form cysts once the supply of prey organism has been depleted.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Dileptus americanus* Kahl (ATCC 50259)

References

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

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