**Nuclearia sp.** (ATCC® 50694™)

### Storage Temp.
- Frozen: -70°C or colder for 1 week, vapor phase of liquid nitrogen for long-term storage.

### Biosafety Level
- Level 1

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**Please read this FIRST**

**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.

**Culture Maintenance**

1. Harvest cells from a culture that is at or near peak density by centrifugation at 600 x g for 5 min. Pool the cell pellets into a single tube.
2. Adjust the concentration of cells to 2.0 x 10^6/ml. If the concentration is too low, centrifuge at 600 x g for 5 minutes and resuspend the cell pellet with a volume of supernatant to yield the desired concentration.
3. Prepare a 20% (v/v) sterile DMSO solution in fresh medium w/o bacteria, as follows: Add the required volume of DMSO to a glass screw-capped test tube and place on ice. Allow the DMSO to solidify. Add the required volume of refrigerated ATCC medium 802 w/o bacteria. Dissolve the DMSO by inverting several times. If the DMSO solution is not prepared on ice, an exothermic reaction will occur that may precipitate certain components of the medium.
4. Mix the cell preparation and the DMSO in equal portions. Thus, the final concentration will be 10% and 10% (v/v) DMSO. The time from the mixing of the cell preparation and DMSO stock solution before the freezing process is begun should be no less than 15 min and no longer than 60 min.
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.

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**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: Nuclearia sp. (ATCC® 50694™)

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**Description**

**Strain Designation:** CDC

**Deposited Name:** Nuclearia sp.

**Depositor:** TK Sawyer

**Isolation:**
water sample, Atlanta, GA, 1997

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**Propagation**

**Growth Conditions**

Temperature: 25°C

**Medium**

ATCC® Medium 802: Sonneborn's Paramecium medium

**Instructions for Complete Medium**

ATCC Medium 802 inoculated with Klebsiella pneumoniae subsp. pneumoniae (ATCC® 700831™) or Enterobacter aerogenes (ATCC® 13048™).

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**Protocols**

**Cryopreservation**

1. To thaw a frozen ampule, place it in a 35°C water bath, until thawed (2-3 min). Immerse the ampule enough to cover only the frozen material. Do not agitate the ampule.
2. Immediately after thawing, aseptically transfer contents to a plastic screw-capped tube containing 5 ml ATCC medium 802 bacterized with Klebsiella pneumoniae subsp. pneumoniae (ATCC® 700831) or Enterobacter aerogenes (ATCC® 13048). Incubate the tube on a 15° horizontal slant at 25°C with the cap screwed on tightly.

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**Enterobacter aerogenes**

ATCC® 13048™

**Klebsiella pneumoniae**

subsp. pneumoniae (ATCC® 700831™)

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**Enterobacter aerogenes**

ATCC® 13048™

**Klebsiella pneumoniae**

subsp. pneumoniae (ATCC® 700831 or ATCC® 700831™)

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**Enterobacter aerogenes**

ATCC® 13048™

**Klebsiella pneumoniae**

subsp. pneumoniae (ATCC® 700831 or ATCC® 700831™)

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**Enterobacter aerogenes**

ATCC® 13048™

**Klebsiella pneumoniae**

subsp. pneumoniae (ATCC® 700831 or ATCC® 700831™)
7. The frozen preparations are stored in either the vapor or liquid phase of a nitrogen refrigerator.
8. To establish a culture from the frozen state place an ampule in a water bath set at 35°C. Immerse the vial enough to cover only the frozen material. Do not agitate the vial.
9. Immediately after thawing, do not leave in the water bath, aseptically remove the contents of the ampule and inoculate into 5.0 ml of fresh ATCC medium 802 bacterized with Klebsiella pneumoniae subsp. pneumoniae (ATCC® 700831 or Enterobacter aerogenes (ATCC® 13048). Incubate the tube on a 15° horizontal at 25°C with the cap screwed on tightly.

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

Biosafety Level: 1

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.

ATCC Warranty

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Disclaimers

This product is intended for laboratory research purposes only. It is not intended for use in humans.

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

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