



# *Naegleria italica* De Jonckheere

PRA-152™

## Description

**Strain designation:** N 243

**Deposited As:** *Naegleria italica* De Jonckheere

**Type strain:** No

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## Storage Conditions

**Product format:** Frozen

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

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## 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.

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## 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 submerged 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 submerged in liquid nitrogen.

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## Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at [www.atcc.org](http://www.atcc.org).

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## Growth Conditions

**Medium:**

ATCC Medium 997: Fresh water ameba medium

**Instructions for complete medium:** ATCC Medium 997 grown with mixed bacteria

**Temperature:** 25°C

**Culture system:** Xenic

**Incubation:** Non-nutrient agar with *E. coli*

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## 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  $-70^{\circ}\text{C}$  for approximately one week. **Do not under any circumstance store frozen ampules at refrigerator freezer temperatures (generally  $-20^{\circ}\text{C}$ ).** 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  $35^{\circ}\text{C}$  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 the entire contents to a plate of ATCC medium 997. Distribute the material evenly over the plate using a spread bar.
3. Wrap the entire edge of the plate with parafilm and incubate upright at  $25^{\circ}\text{C}$ . Trophozoites should be seen within 2-3 d.

#### **Culture maintenance:**

1. Remove an agar block ( $\sim 5\text{ mm}^2$ ), with trophozoites or cysts, from the edge of an agar plate culture and place it in a test tube containing 1 ml of sterile ATCC<sup>®</sup> medium 1323. Agitate to suspend cells from the agar block. Transfer 0.25 ml of the solution to center of each of two fresh plates and spread evenly with a spread bar.
2. Wrap the entire edge of the plate with parafilm and incubate upright at  $25^{\circ}\text{C}$ .
3. Repeat steps 1-3 at 10-14 d intervals.

#### **Cryopreservation:**

1. Allow the cells to encyst. To detach cysts from the plate flush the surface with 5 ml fresh ATCC<sup>®</sup> medium 1323 (Page's Balanced Salt Solution). Rub the surface of the plate with a spread bar to detach adhering amoebae.
2. Transfer the cyst suspension to a sterile centrifuge tube.
3. If the cyst concentration does not exceed  $2 \times 10^6$  cysts/ml adjust the suspension to that concentration. To adjust the concentration, centrifuge at  $600 \times g$  for 5 min and resuspend the pellet in the volume of fresh medium required to yield  $2 \times 10^6$ .
4. While cells are centrifuging prepare a 15% (v/v) solution of sterile DMSO as follows: Add the required volume of DMSO to a glass screw-capped test tube and place it in an ice bath. Allow the DMSO to solidify. Add the required volume of refrigerated medium. Dissolve the DMSO by inverting the tube several times.

## **Material Citation**

If use of this material results in a scientific publication, please cite the material in the following manner: *Naegleria italica* De Jonckheere (ATCC PRA-152)

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## **References**

References and other information relating to this material are available at [www.atcc.org](http://www.atcc.org).

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## Revision

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## ***Naegleria italica* De Jonckheere**

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