# *Colpoda* sp.

## PRA-118™

### Description

**Strain designation:** 01-RI-22
**Type strain:** No

### Storage Conditions

**Product format:** Dried

### 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 2348: Freshwater Diplophrys 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).
**Temperature:** 15°C
**Incubation:** Grown with Enterobacter aerogenes ATCC 13048 as food source. Dilute ATCC medium 802 in ATCC medium 2348 at a 1:5 ratio

## Handling Procedures

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 ampules 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.

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. Incubate with the cap tightly sealed at 25°C.

**Culture maintenance:**
The culture will encyst over time. To facilitate excystation, add wheat grains to the flask (1 grain for a T25, or 3 grains for a T75). Subculture every two weeks to a fresh T-25 flask of bacterized medium in the following manner:

1. Scrape the flask bottom using a sterile cell scraper and aseptically transfer 0.5 ml from a growing culture to a T-25 tissue culture flask containing 9.5 ml of ATCC medium 802 bacterized with *Klebsiella pneumoniae* subsp. *pneumoniae* (ATCC® 700831) or *Enterobacter aerogenes* (ATCC® 13048).

2. Incubate flask at 25°C with the cap screwed on tightly.

**Reagents for cryopreservation:**
Cryoprotective Solution

| | |
|---|---|
| DMSO | 2.0 ml |
| Fresh growth medium w/o bacteria | 8.0 ml |

**Cryopreservation:** 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 cells from a culture that is at or near peak density by centrifugation at 800 x g for 5 min. Culture should consist primarily of cysts, as the trophozoites do not survive the freeze well. To encourage encystation, wheat grains can be removed

from the flask a few days prior to harvesting.

3. Adjust the concentration of cells to at least 2 x $10^6$/ml in fresh medium.

4. Mix the cell preparation and the cryoprotective solution in equal portions. Final DMSO concentration will be 10%.

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 3 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 ATCC medium 802 bacterized with *Klebsiella pneumoniae* subsp. *pneumoniae* (ATCC® 700831) or *Enterobacter aerogenes* (ATCC® 13048).

9. Incubate at 25°C with the cap screwed on tightly.

10. Once the culture is established, vigorously agitate or scrape the flask and aseptically transfer 0.5 ml to 10.0 ml of bacterized ATCC medium 802.

11. Follow the protocol for maintenance of culture.

## Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Colpoda* sp. (ATCC PRA-118)

## References

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

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## Revision

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