



Product Sheet

# *Crithidia brachyflagelli* (ATCC® PRA-345™)

Please read this FIRST

Storage Temp.  
**Frozen Cultures:**  
-70°C for 1 week;  
liquid N<sub>2</sub> vapor  
for long term  
storage



**Freeze-dried Cultures:**  
2-8°C

**Live Cultures:**  
See Protocols  
section for  
handling  
information



Biosafety Level  
1

## 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: *Crithidia brachyflagelli* (ATCC® PRA-345™)

American Type Culture Collection  
PO Box 1549  
Manassas, VA 20108 USA  
[www.atcc.org](http://www.atcc.org)

800.638.6597 or 703.365.2700  
Fax: 703.365.2750  
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## Description

**Strain Designation:** 340VL

**Depositor:** D Maslov

**Isolation:** Intestine of undetermined mirid (Miridae, Heteroptera), Puntarenas Province, Costa Rica, 2009



## Propagation

### Growth Conditions

**Temperature:** 20°C to 25°C

### Medium

ATCC® Medium 44: Brain Heart Infusion Agar/Broth

### Instructions for Complete Medium

**Media:** ATCC medium 44 supplemented with 10 µg/mL hemin



## Protocols

### 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 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 in a 35°C water bath, until thawed (2-3 min). Immerse the ampule just sufficiently to cover the frozen material. Do not agitate the ampule.
2. Immediately after thawing, aseptically transfer contents to a T-25 tissue culture flask containing 10.0 mL ATCC medium 44 supplemented with 10 µg/mL hemin. Incubate at 20-25°C with the cap screwed on tightly.

### Culture Maintenance

1. Agitate a culture at or near peak density and aseptically transfer 0.1-0.2 mL to a fresh flask of ATCC medium 44 supplemented with 10 µg/mL hemin.
2. Incubate at 20-25°C with the cap screwed on tightly.
3. Transfer the culture every 7-10 days as described in steps 1-2. The transfer interval will depend on the quantity of the inoculum and the quality of the medium. This should be empirically determined by examining the culture on a daily basis until the growth cycle has stabilized.



## Cryopreservation

### Harvest and Preservation

1. Harvest cells from a culture which is at or near peak density by centrifugation at ~800 x g for 5 min.
2. Adjust concentration of cells to 2 x 10<sup>7</sup>/mL in fresh medium. If the concentration is too low, centrifuge at ~800 x g for 5 minutes and resuspend the cell pellet with a volume of supernatant to yield the desired concentration.
3. While cells are centrifuging, prepare a 10% (v/v) solution of sterile DMSO in fresh medium (broth). The DMSO solution when first prepared will warm up due to chemical heat. The solution should be allowed to return to room temperature prior to use.
4. Mix the cell preparation and the DMSO solution in equal portions. The final concentration will be 10<sup>7</sup> cells/mL and 5% (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 more than 30 min.
5. Dispense in 0.5 mL aliquots into 1.0 - 2.0 mL sterile plastic screw-capped cryovials (special plastic vials for cryopreservation).
6. Place the ampules 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.) 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.
7. Store in either the vapor or liquid phase of a nitrogen refrigerator.
8. 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.



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9. Remove the vial from the water bath immediately after thawing. Aseptically transfer the contents of the ampule into a T-25 tissue culture flask containing 10.0 mL ATCC medium 44 supplemented with 10 µg/mL hemin.
10. Incubate the tube at 20-25°C with the cap screwed on tightly.
11. Maintain as described above.



## References

References and other information relating to this product are available online at [www.atcc.org](http://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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Additional information on this culture is available on the ATCC web site at [www.atcc.org](http://www.atcc.org).

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