



Product Sheet

Toxoplasma gondii (ATCC® 50174™)

Please read this FIRST

Storage Temp.
Frozen Cultures:
-70°C for 1 week;
liquid N₂ vapor
for long term
storage



Freeze-dried Cultures:
2-8°C

Live Cultures:
See Protocols
section for
handling
information



Biosafety Level
2

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: *Toxoplasma gondii* (ATCC® 50174™)

American Type Culture Collection
PO Box 1549
Manassas, VA 20108 USA
www.atcc.org

800.638.6597 or 703.365.2700
Fax: 703.365.2750
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Or contact your local distributor

Description

Strain Designation: RH
Deposited Name: *Toxoplasma gondii* (Nicolle and Manceaux) Nicolle and Manceaux
Depositor: RG Yaeger
Isolation: 6-year-old male Homo sapiens, Cincinnati, OH, 1939
Genotype: Haplogroup 1

Propagation

Growth Conditions
Culture System: *In vivo* cultivation, mouse

Protocols

Storage and Culture Initiation

Frozen ampoules 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 ampoules 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. Immediately after thawing, aseptically remove the contents of the ampule with a syringe and inoculate an uninfected mouse. Follow the protocol for maintenance *in vivo*. The course of infection may be longer or shorter than usual depending on percent recovery of the parasite from the frozen state.

Culture Maintenance

Tyrod's Salt Solution

NaCl, 8.00 g
KCl, 0.20 g
CaCl₂ 0.20 g
MgCl₂ • H₂O 0.05 g
NaH₂PO₄ • H₂O 1.00 g
NaHCO₃ • H₂O 1.00 g
Glucose 1.00 g
Glass distilled H₂O to 1.00 L

Add ingredients in the sequence listed. Filter-sterilize.

1. Inject the entire contents of the thawed ampule intraperitoneally into a 6- to 9-week-old mouse. The infection should be well developed within 6-9 days post inoculation. The abdomen of the mouse will become increasingly swollen as the infection progresses.
2. When the infection is well developed, remove the peritoneal fluid from the mouse using the following technique:
 - a. Inject 2 mL of Tyrod's solution into the peritoneum; massage the stomach for 2 minutes, and then using a 5-mL syringe, remove the peritoneal fluid (~3 mL).
 - b. Inoculate 0.2 mL of this fluid per mouse to subculture.

Cryopreservation

Harvest and Preservation

1. Harvest the parasites according to the protocol for maintenance *in vivo*.
2. Spin the cell suspension at approximately 50 x g for 3 min, to remove the cellular debris.
3. Transfer the supernatant to a new 15 mL plastic centrifuge tube. Centrifuge at 1300 x g for 10 min.
4. Pool the cell pellets and adjust the concentration to 2.0 - 4.0 x 10⁷ cells/mL with a fresh solution of Tyrod's Salt Solution.
*If the concentration is too low centrifuge at 1300 x g for 10 min and resuspend in the volume of Tyrod's Salt Solution required to yield the desired concentration.
5. Mix the cell preparation and 15% (v/v) DMSO in equal portions. The final concentration will be 1.0 - 2.0 x 10⁷ cells/mL and 7.5% DMSO. The time from the mixing of the cell preparation and cryoprotective solution to the start of the freezing process should be no less than 15 min. and no




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- more than 30 min.
6. Dispense in 0.5 mL aliquots to 1.0-2.0 mL sterile plastic screw-capped cryules (special plastic vials for cryopreservation).
 7. Place the vials in a controlled rate freezing unit. From room temperature cool at -1°C/min to -40°C. If the freezing unit can compensate for the heat of fusion, maintain rate at -1°C/min through the heat of fusion. At -40°C plunge 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.)
 8. Store in either the vapor or liquid phase of a nitrogen refrigerator.
 9. 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 thawed.
 10. Immediately after thawing, aseptically remove the contents of the ampule with a syringe and inoculate an uninfected mouse. Follow the protocol for maintenance *in vivo*.

 **References**

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

 **Biosafety Level: 2**

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.

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Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at www.atcc.org

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