

***Toxoplasma gondii*, Strain Pru A7  
 $\Delta$ hxgprt::*gra2-GFP::tub1-FLUC***

**Catalog No. NR-49335**

**For research use only. Not for human use.**

**Contributor:**

John Boothroyd, Professor, Department of Microbiology and Immunology, Stanford University, Stanford, California, USA

**Manufacturer:**

BEI Resources

**Product Description:**

Protozoa Classification: *Apicomplexa*, *Toxoplasma*

Species: *Toxoplasma gondii*

Strain: Pru A7  $\Delta$ hxgprt::*gra2-GFP::tub1-FLUC (also referred to as Pru A7  $\Delta$ hpt::*gra2-GFP::tub1-FLUC)<sup>1</sup>**

Original Source: *Toxoplasma gondii* (*T. gondii*), strain Pru A7  $\Delta$ hxgprt::*gra2-GFP::tub1-FLUC was deposited to BEI Resources as a green-fluorescent-protein- and luciferase-expressing transgenic clone derived from the deletion mutant strain Prugniaud (Pru)  $\Delta$ hxgprt (also referred to as Pru  $\Delta$ hpt), which lacks the *hxgprt* (hypoxanthine-xanthine-guanine-phosphoribosyltransferase) gene.<sup>1</sup> The original parent strain Prugniaud (Pru) is a Type II strain originally isolated in 1964 from a human with lethal congenital toxoplasmosis in Limoges, France.<sup>2</sup>*

Comment: *T. gondii*, strain Pru A7  $\Delta$ hxgprt::*gra2-GFP::tub1-FLUC was engineered by transfection of the Pru  $\Delta$ hxgprt strain with a plasmid expressing GFP and FLUC under the control of the *Toxoplasma* GRA2 and TUB1 promoters, respectively, resulting in constitutive expression of GFP and FLUC in both tachyzoite and bradyzoite stages of the parasite.<sup>1</sup> Strain Pru A7  $\Delta$ hxgprt::*gra2-GFP::tub1-FLUC has been used to study the role of bradyzoite-specific antigens in establishing infections.<sup>1</sup>**

*T. gondii* is an obligate intracellular protozoan parasite of the phylum *Apicomplexa* that is the causal agent of toxoplasmosis. *T. gondii* has a highly unusual, clonal population structure comprised of three widespread genotypes referred to as type I (highly virulent), type II (nonvirulent), and type III (associated with animal infections), which account for >95% of strains isolated in North America and Europe.<sup>3-6</sup> Isolates from South America exhibit greater genetic diversity. Phylogenetic analyses of *T. gondii* intron sequences have identified eleven separate haplogroups, with striking geographic separation between North America, Europe, and South America.<sup>7</sup>

**Material Provided:**

Each vial of NR-49335 contains approximately 0.5 mL of culture in cryopreservative [7.5% dimethylsulfoxide (DMSO)]. Please refer to the Certificate of Analysis for the specific culture media used for each lot and refer to Appendix I for cryopreservation instructions.

**Packaging/Storage:**

NR-49335 was packaged aseptically in screw-capped plastic cryovials and is provided frozen on dry ice. The product should be stored at cryogenic temperature (-130°C or colder), preferably in the vapor phase of a liquid nitrogen freezer. If liquid nitrogen storage facilities are not available, frozen cryovials may be stored at -70°C or colder for approximately one week.

Note: Do not under any circumstances store vials at temperatures warmer than -70°C. Storage under these conditions will result in the death of the culture.

To insure the highest level of viability, the culture should be initiated immediately upon receipt. Any warming of the product during shipping and transfer must be avoided, as this will adversely affect the viability of the product. For transfer between freezers and for shipping, the product may be placed on dry ice for brief periods, although use of a portable liquid nitrogen carrier is preferred. Please read the following recommendations prior to using this material.

**Growth Conditions:**

ATCC® medium 2222: Cell cultivation medium for parasites (Dulbecco's Minimal Essential Medium), adjusted to contain 10% (v/v) heat-inactivated fetal bovine serum (HIFBS)

Human foreskin fibroblast cells (ATCC® CRL-1634™)

Incubation:

Temperature: 35°C to 37°C

Atmosphere: Aerobic with 5% CO<sub>2</sub>

Propagation:

1. To establish a culture from the frozen state, place a vial in a 35°C to 37°C water bath. Thawing time is approximately 2 to 3 minutes. Do not agitate the vial. Do not leave the vial in the water bath after it is thawed.
2. Immediately after thawing, aseptically transfer the contents to a tissue culture flask containing a fresh monolayer of human foreskin fibroblast cells (ATCC® CRL-1634™) and 10 mL of ATCC® medium 2222 containing 10% (v/v) HIFBS.
3. Outgas the flask for 10 seconds with a 95% air, 5% CO<sub>2</sub> gas mixture.
4. Incubate in a 35°C to 37°C CO<sub>2</sub> incubator with the caps screwed on tightly. Observe the culture daily under an inverted microscope for the presence of parasitophorous vacuoles.

Maintenance:

1. Remove the medium from a fresh confluent monolayer of human foreskin fibroblast cells in a tissue culture flask and replace it with 10 mL medium containing 10% (v/v) HIFBS.
2. Remove the medium from the *Toxoplasma* culture when approximately 50% of the human foreskin fibroblast cell monolayer has lysed. Centrifuge the parasites that had been released into the medium at 1300 x g for 10 minutes.
3. Remove the supernatant and resuspend the cell pellet in a small volume (0.5 mL to 1.0 mL) of ATCC® medium 2222 or phosphate buffered saline (PBS). Transfer the resuspended pellet to the fresh flask of human foreskin

fibroblast cells, prepared in step 1 above. Follow steps 3 and 4 in Propagation.

**Citation:**

Acknowledgment for publications should read “The following reagent was obtained through BEI Resources, NIAID, NIH: *Toxoplasma gondii*, Strain Pru A7  $\Delta$ hxgprt::gra2-GFP::tub1-FLUC, NR-49335.”

**Biosafety Level: 2**

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories. 5th ed. Washington, DC: U.S. Government Printing Office, 2009; see [www.cdc.gov/biosafety/publications/bmbl5/index.htm](http://www.cdc.gov/biosafety/publications/bmbl5/index.htm).

**Disclaimers:**

You are authorized to use this product for research use only. It is not intended for human use.

Use of this product is subject to the terms and conditions of the BEI Resources Material Transfer Agreement (MTA). The MTA is available on our Web site at [www.beiresources.org](http://www.beiresources.org).

While BEI Resources uses reasonable efforts to include accurate and up-to-date information on this product sheet, neither ATCC® nor the U.S. Government makes any warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. Neither ATCC® nor the U.S. Government warrants that such information has been confirmed to be accurate.

This product is sent with the condition that you are responsible for its safe storage, handling, use and disposal. ATCC® and the U.S. Government are not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to ensure authenticity and reliability of materials on deposit, the U.S. Government, ATCC®, their suppliers and contributors to BEI Resources are not liable for damages arising from the misidentification or misrepresentation of products.

**Use Restrictions:**

**This material is distributed for internal research, non-commercial purposes only.** This material, its product or its derivatives may not be distributed to third parties. Except as performed under a U.S. Government contract, individuals contemplating commercial use of the material, its products or its derivatives must contact the contributor to determine if a license is required. U.S. Government contractors may need a license before first commercial sale.

**References:**

1. Kim, S. K., et al. “Bradyzoite-Specific Surface Antigen SRS9 Plays a Role in Maintaining *Toxoplasma gondii* Persistence in the Brain and in Host Control of Parasite

Replication in the Intestine.” Infect. Immun. 75 (2007): 1626-1634. PubMed: 17261600.

2. Darde, M. L., et al. “Isoenzyme Analysis of 35 *Toxoplasma gondii* Isolates and the Biological and Epidemiological Implications.” J. Parasitol. 8 (1992): 786-794. PubMed: 1403418.

3. Howe, D. K. and L. D. Sibley. “*Toxoplasma gondii* Comprises Three Clonal Lineages: Correlation of Parasite Genotype with Human Disease.” J. Infect. Dis. 172 (1995): 1561-1566. PubMed: 7594717.

4. Sibley, L. D. and J. C. Boothroyd. “Virulent Strains of *Toxoplasma gondii* Comprise a Single Clonal Lineage.” Nature 359 (1992): 82-85. PubMed: 1355855.

5. Khan, A. et al. “Composite Genome Map and Recombination Parameters Derived from Three Archetypal Lineages of *Toxoplasma gondii*.” Nucleic Acids Res. 33 (2005): 2980-2992. PubMed: 15911631.

6. Sibley, L. D., et al. “Generation of a Restriction Fragment Length Polymorphism Linkage Map for *Toxoplasma gondii*.” Genetics 132 (1992): 1003-1015. PubMed: 1360931.

7. Khan, A., et al. “Recent Transcontinental Sweep of *Toxoplasma gondii* Driven by a Single Monomorphic Chromosome.” Proc. Natl. Acad. Sci. USA 104 (2007): 14872-14877. PubMed: 17804804.

8. Boothroyd, J. C. “Have it Your Way: How Polymorphic, Injected Kinases and Pseudokinases Enable *Toxoplasma* to Subvert Host Defenses.” PLoS Pathog. 4 (2013): e1003296. PubMed: 23633947.

ATCC® is a trademark of the American Type Culture Collection.



**APPENDIX I: CRYOPRESERVATION**

1. To harvest the *Toxoplasma* culture, detach any remaining tissue culture cells (infected and uninfected) by scraping the surface of the flask with a cell scraper.
2. Transfer the cell suspension (including parasites) to 15 mL plastic centrifuge tubes. Centrifuge at 1300 × g for 10 min.
3. Remove all but 0.5 mL of the supernatant from each tube, resuspend the cell pellets, and pool them to a single tube.
4. Pass the resulting cell suspension through a syringe equipped with a 27-gauge ½-inch needle to break up any remaining cells.
5. Adjust the parasite concentration to  $2 \times 10^7$  to  $4 \times 10^7$  cells/mL with fresh medium [Cell cultivation medium for parasites (ATCC® medium 2222) or Dulbecco's PBS (ATCC® 30-2200) can be used].  
Note: If the concentration of parasites is too low, centrifuge at 1300 x g for 10 min and resuspend in a smaller volume of fresh medium to yield the desired parasite concentration.
6. Mix equal volumes of parasite suspension and fresh medium or PBS containing 15% DMSO and 50% HIFBS to yield a final concentration of  $1 \times 10^7$  to  $2 \times 10^7$  cells/mL in 7.5% DMSO, 25% HIFBS. The freezing process should start 15 to 30 minutes following the addition of cryoprotective solution to the parasite suspension.  
Note: To prevent culture contamination, penicillin-streptomycin solution (ATCC® 30-2300) may be added to a final concentration of 50 U/mL to 100 U/mL penicillin and 50 µg/mL to 100 µg/mL streptomycin.
7. Dispense 0.5 mL aliquots into 1 mL to 2 mL sterile plastic screw-capped vials for cryopreservation.
8. Place the vials in a controlled rate freezing unit. From room temperature cool the vials 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 this phase. At -40°C, plunge vials into liquid nitrogen. Alternatively, place the vials in a Nalgene 1°C freezing container. Place the container at -80°C for 1.5 to 2 hours and then plunge vials into liquid nitrogen.
9. Store in either the vapor or liquid phase of a nitrogen refrigerator (-130°C or colder).