# *Toxoplasma gondii*, Clone c285-62

## Catalog No. NR-10267

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

### Contributor:

L. David Sibley, Professor, Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, Missouri.

### Product Description:

Protozoa Classification: *Apicomplexa*, *Toxoplasma*
Species: *Toxoplasma gondii*
Clone: c285-62
Comment: *Toxoplasma gondii* (*T. gondii*), c285-62 is a recombinant F1 clone of intermediate virulence selected from progeny of two parallel genetic crosses between a highly virulent Type I parental strain (GT1-FUDR3.3; BEI Resources NR-10272) and the non-virulent Type III parental strain (CTG.11 ARA-SNF; also referred to as CTG.11 ARA-$A^R$/$SNF^R$ and CEP.11 ARA-$A^R$/$SNF^R$, BEI Resources NR-10273).[1,2] *T. gondii*, CTG ARA-SNF is available as BEI Resources NR-10151.

*T. gondii*, c285-62 was deposited at BEI Resources as a recombinant clone of intermediate virulence. The c285-62 clone is fully resistant *in vitro* to arabinoside (ara-A), 5-fluoro-2-deoxy uridine (FUDR) and sinefungin (SNF) at levels that fully inhibit growth of wild type *Toxoplasma* strains.[1,2]

*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, type II, and type III. Type I are the highly virulent, type II are nonvirulent and give rise to chronic infections in humans, and type III are also non-virulent but mainly associated with chronic infections in animals.[3,4] The genetic basis for the differences between the three lineages has been recently examined by mapping virulence in F1 progeny derived from crosses between the different *T. gondii* lineages.[5,6]

### Material Provided:

Each vial of NR-10267 contains approximately 0.5 mL of culture in cryopreservative. Please see Appendix I below for cryopreservation instructions.

### Packaging/Storage:

NR-10267 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 to 37°C
Atmosphere: 95% air, 5% $CO_2$
Propagation:

1. To establish a culture from the frozen state, place a vial in a 35 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% $CO_2$ gas mixture.
4. Incubate in a 35 to 37°C $CO_2$ 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 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 the NIH Biodefense and



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Emerging Infections Research Resources Repository, NIAID, NIH: *Toxoplasma gondii*, Clone c285-62, NR-10267."

## 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, 2007; see www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm.

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## References:

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## 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 x 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 2.0 to 4.0 x $10^7$ 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 20% DMSO and 50% HIFBS to yield a final concentration of 1.0 to 2.0 x $10^7$ cells/mL in 10% 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 to 100 U/mL penicillin and 50 to 100 µg/mL streptomycin.
7. Dispense 0.5 mL aliquots into 1 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).



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