Product Information Sheet for NR-51144

Toxoplasma gondii, Strain RH Δ*rop17* Δ*rop18*

Catalog No. NR-51144

For research use only. Not for human use.

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Manufacturer:
BEI Resources

Product Description:
Protozoa Classification: *Apicomplexa*, *Toxoplasma*
Species: *Toxoplasma gondii*
Strain: RH Δ*rop17* Δ*rop18*
Original Source: *Toxoplasma gondii (T. gondii)*, strain RH Δ*rop17* Δ*rop18* was deposited to BEI Resources as a mutant of the virulent Type I strain RH created by the deletion of the *rop17* and *rop18* loci.[1]

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.[2-5] 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.[6]

T. gondii manipulates host functions through a family of protein kinases and pseudokinases injected into the host cell from apical secretory organelles called rhoptries (ROP).[7] ROP kinases are polymorphic and account for much of the difference in virulence between different strains of *Toxoplasma*.[7] The ROP17 kinase targets threonine residue 102 in the host immunity related GTPase (IRG) Irgb6.[1] ROP17 acts synergistically with ROP18 to block the host IRG pathway and control acute virulence of *T. gondii* in mice.[1] ROP18 is a well-studied serine-threonine kinase that phosphorylates and inactivates host interferon-gamma-inducible p47 GTPases.[8,9] ROP18 has also been reported to bind and inactivate ATF6β, a host transcription factor with a role in the IFN-γ response.[10]

Material Provided:
Each vial of NR-51144 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-51144 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_2
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_2 gas mixture.
4. Incubate in a 35°C 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 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

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

Please refer to Appendix I for cryopreservation instructions.

Citation:

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: *Toxoplasma gondii*, Strain RH Δ*rop17* Δ*rop18*, NR-51144."

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.

Disclaimers:

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References:

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7. 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.
8. Fentress, S. J., et al. "Phosphorylation of Immunity-Related GTPases by a *Toxoplasma gondii*-Secreted Kinase Promotes Macrophage Survival and Virulence." Cell Host Microbe 8 (2010): 484-495. PubMed: 21147463.
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10. Yamamoto, M., et al. "ATF6β Is a Host Cellular Target of the *Toxoplasma gondii* Virulence Factor ROP18." J. Exp. Med. 208 (2011): 1533-1546. PubMed: 21670204.

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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 × 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 2×10^7 to 4×10^7 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 × 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 1×10^7 to 2×10^7 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).

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