Toxoplasma gondii, 2F (RH-2F)

Catalog No. NR-224

(Derived from ATCC® 50839™)

For research use only. Not for human use.

Contributor:

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Product Description:

Protozoa Classification: *Sarcocystidae, Toxoplasma*
Species: *Toxoplasma gondii*
Strain: 2F (RH-2F)
Source:[1] *Toxoplasma gondii* 2F (RH-2F) is an RH-88 transformant harboring the *Escherichia coli lacZ* gene driven by the GRA1 promoter. The RH-88 strain was derived from the RH strain, which was isolated in 1939 from a six year old boy with a lethal case of encephalitis in Cincinnati, Ohio.[2]
Comment: The 2F (RH-2F) strain was deposited to the ATCC® in 2000 by Dr. L. David Sibley of the Washington University School of Medicine in St. Louis, Missouri, as Type I genotype: SAG1-1; SAG2-1; SAG3-1.

Toxoplasma gondii is a protozoan parasite member of the phylum *Apicomplexa* and an obligate intracellular pathogen that is the causal agent of toxoplasmosis. It is highly amendable to experimental manipulation in the laboratory, and serves as a model system for genetic exploration of parasite biology and host-parasite interactions.[3]

Material Provided:

Each vial of NR-224 contains approximately 0.5 mL of culture in cryopreservative. Please see Appendix I below for cryopreservation instructions.

Packaging/Storage:

NR-224 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:

Growth Media:
ATCC medium 2222: Cell cultivation medium for parasites, adjusted to contain 3% (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 and 10 mL of growth medium containing 3% (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 until peak density is achieved.

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 of growth medium containing 3% (v/v) HIFBS.
2. To transfer the *Toxoplasma* culture, remove the old medium containing the organism and centrifuge 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 growth medium 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 Emerging Infections Research Resources Repository, NIAID, NIH: *Toxoplasma gondii*, 2F (RH-2F), NR-224."

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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3. Kim, K. and L. M. Weiss. "*Toxoplasma gondii*: The Model Apicomplexan." Int. J. Parasitol. 34 (2004): 423-432. PubMed: 15003501.
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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 1.0-2.0 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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