Product Information Sheet for NR-53930

Toxoplasma gondii, Strain EGS SAG1-mCherry LDH2-sfGFP

Catalog No. NR-53930

For research use only. Not for use in humans.

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Manufacturer:
BEI Resources

Product Description:
Protozoa Classification: *Apicomplexa*, *Toxoplasma*
Species: *Toxoplasma gondii*
Strain: EGS SAG1-mCherry LDH2-sfGFP
Original Source: *Toxoplasma gondii (T. gondii),* strain EGS SAG1-mCherry LDH2-sfGFP was deposited to BEI Resources as a transgenic strain that expresses the red fluorescent protein mCherry and superfolder green fluorescent protein (sfGFP) at specific stages of the parasite's life cycle.[1,2,3] Strain EGS SAG1-mCherry LDH2-sfGFP was derived from the recombinant type I/III strain EGS, isolated in 1998 from amniotic fluid of a human patient with congenital toxoplasmosis in Brazil, that is highly virulent in mice and is able to spontaneously form cysts *in vitro*.[4,5,6,7]
Comments: *T. gondii,* strain EGS SAG1-mCherry LDH2-sfGFP was engineered by transfection of the EGS strain with plasmids expressing mCherry and sfGFP under the control of stage-specific promoters SAG-1 (tachyzoite-specific) and LDH-2 (bradyzoite-specific), respectively.[2,3] Strain EGS SAG1-mCherry LDH2-sfGFP parasites in the bradyzoite stage are positive for sfGFP and negative for mCherry. Parasites in the tachyzoite stage are positive for mCherry and negative for sfGFP. Expression of the two fluorescent proteins at distinct stages of the life cycle provides a useful tool to elucidate the mechanisms of *T. gondii* differentiation *in vitro* and *in vivo*.

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.[8,9,10,11] 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.[12]

Life cycle stages of *T. gondii* include sporozoites, merozoites, tachyzoites and bradyzoites. The tachyzoite form may convert into the long term bradyzoite form under certain conditions such as the host immune response.[3]

Material Provided:
Each vial of NR-53930 contains approximately 0.5 mL of culture in cryopreservative [7.5% dimethylsulfoxide (DMSO)]. Please refer to Appendix I for cryopreservation instructions.

Packaging/Storage:
NR-53930 was packaged aseptically in screw-capped plastic cryovials and is provided frozen on dry ice. The product should be stored at -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 ensure 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:
Dulbecco's Minimal Essential Medium (DMEM), adjusted to contain 10% (v/v) heat-inactivated fetal bovine serum (HIFBS)
Note: Optimal expression of sfGFP in bradyzoite cysts may be achieved by adjusting the growth medium to pH 8.[3]
Human foreskin fibroblast cells (ATCC® CRL-1634™)
Incubation:
Temperature: 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 vented-cap tissue culture flask containing a fresh monolayer of human foreskin fibroblast cells (ATCC® CRL-1634™) and 10 mL of DMEM containing 10% (v/v) HIFBS.
3. Incubate at 37°C in an aerobic atmosphere with 5% CO_2. 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 × 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 DMEM containing 10% (v/v) HIFBS 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.

Note: Not all parasites display fluorescence. Silencing may occur in the expression of these markers due to epigenetic effects or to loss of the integrated plasmids. It is recommended to maintain cultures at a low passage to avoid plasmid loss. Fluorescent-activated cell sorting (FACS) may be necessary to eliminate low expression variants.[1,3]

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 EGS SAG1-mCherry LDH2-sfGFP, NR-53930."

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. 6th ed. Washington, DC: U.S. Government Printing Office, 2020; see www.cdc.gov/biosafety/publications/bmbl5/index.htm.

Disclaimers:
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Support Provided by NIAID

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 minutes.
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 per mL with fresh medium [DMEM containing 10% (v/v) HIFBS 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 minutes 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 per 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 IU per mL to 100 IU per mL penicillin and 50 µg per mL to 100 µg per 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 per minute to -40°C. If the freezing unit can compensate for the heat of fusion, maintain rate at -1°C per 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).