Trypanosoma cruzi, Y Strain (+*luc*)

Catalog No. NR-40347

For research use only. Not for human use.

Contributor:

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Manufacturer:

BEI Resources

Product Description:

Protozoa Classification: *Trypanosomatidae*, *Trypanosoma*
Species: *Trypanosoma cruzi*
Strain: Y Strain (+*luc*)
Original Source: *Trypanosoma cruzi* (*T. cruzi*), Y strain (+*luc*) is a transgenic clone derived from the pathogenic Y strain, which was originally isolated from the blood of a Chagas' disease patient prior to 1953 in Brazil.[1-3]
Comment: *T. cruzi,* Y strain (+*luc*) was deposited to BEI Resources as a luciferase-expressing strain in the trypomastigote stage of the parasite's life cycle. Luciferase was introduced via the pTREX-GFP plasmid where *luc* replaced GFP.[1]
Note: The luciferase expressing plasmid was not integrated into the *T. cruzi* genome. It is recommended that luciferase activity be assayed monthly to confirm plasmid retention.

The protozoan parasite *T. cruzi* is the causative agent of Chagas' disease, a debilitating disease endemic in many Latin American countries. The search for trypanosomicidal compounds can be facilitated by utilization of a bioluminescent reaction to quantitate parasitic growth in cell culture (modeling an *in vivo* infection).

T. cruzi, Y strain (+*luc*) has been genetically engineered to express luciferase (*luc*) an enzyme that emits a bioluminescent signal upon exposure to luciferin and adenosine triphosphate (ATP).[3] The generation of *T. cruzi* parasite lines that express bioluminescent or fluorescent proteins has allowed high throughput screening of large numbers of candidate compounds against the parasite using *in vitro* as well as *in vivo* systems.[4,5]

Material Provided:

Each vial of NR-40347 contains approximately 0.5 mL of culture in cryopreservative. Please see Appendix I for cryopreservation instructions.

Packaging/Storage:

NR-40347 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:

Dulbecco's Modified Eagle's Medium (DMEM) (ATCC® 30-2002™) or Eagle's Minimum Essential Medium (EMEM) (ATCC® 30-2003™) adjusted to contain 10% (v/v) heat-inactivated fetal bovine serum (HIFBS)
BALB/3T3 clone A31 mouse embryonic fibroblasts (ATCC® CCL-163™) or BS-C-1 monkey kidney epithelial cells (ATCC® CCL-26™)
Incubation:
Temperature: 37°C
Atmosphere: 95% air, 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 BALB/3T3 clone A31 mouse embryonic fibroblasts (ATCC® CCL-163™) and 10 mL of growth medium 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. Observe the culture daily under an inverted microscope for the presence of intracellular forms of the parasite. The emergence of trypomastigotes from host cells is usually observed between 5 to 7 days.

Maintenance:

1. Remove the medium from a fresh confluent host cell monolayer in a tissue culture flask and replace it with 10 mL medium containing 10% (v/v) HIFBS.
2. Remove the medium containing the trypomastigotes from the *Trypanosoma* culture and transfer to a 15 mL centrifuge tube. Centrifuge the parasites 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. Transfer the resuspended pellet to the fresh culture flask 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 BEI Resources, NIAID, NIH: *Trypanosoma cruzi*, Y Strain (+*luc*), NR-40347."

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:

1. A. Rodriguez, Personal Communication.
2. Silva, L. H. P. and Nussenzweig, V. "Sobre Uma Cepa de *Trypanosoma cruzi* Altamente Virulenta Para o Camundongo Branco." Folia Clin. Biol. 20 (1953): 191-207.
3. Andriani, G., et al. "Activity *in vivo* of Anti-*Trypanosoma cruzi* Compounds Selected from a High Throughput Screening." PLoS. Negl. Trop. Dis. 5 (2011): e1298. PubMed: 21912715.
4. Hyland, K. V., et al. "Bioluminescent Imaging of *Trypanosoma cruzi* Infection." Int. J. Parasitol. 38 (2008): 1391-1400. PubMed: 18511053.
5. Canavaci, A. M., et al. "*In vitro* and *in vivo* High-Throughput Assays for the Testing of Anti-*Trypanosoma cruzi* Compounds." PLoS. Negl. Trop. Dis. 13 (2010): e740. PubMed: 20644616.

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APPENDIX I: CRYOPRESERVATION

1. To harvest *Trypanosoma cruzi*, remove the media containing trypomastigotes from a series of infected cultures in T75 flasks and transfer 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 into a single tube.
4. Adjust the parasite concentration to 2.0 to 4.0 x 10^7 cells/mL using fresh growth medium.
   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 containing 10% DMSO to yield a final concentration of 1.0-2.0 x 10^7 cells/mL in 5% DMSO. 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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