Trypanosoma cruzi, Strain CL Brener (+*luc*)

Catalog No. NR-49161

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Contributor:

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Manufacturer:

BEI Resources

Product Description:

Protozoa Classification: *Trypanosomatidae*, *Trypanosoma*
Species: *Trypanosoma cruzi*
Strain: CL Brener (+*luc*)
Original Source: *Trypanosoma cruzi (T. cruzi)*, strain CL Brener (+*luc*) is a transgenic clone derived from strain CL Brener.[1,2] Strain CL Brener is a clone of the CL strain, a genotype TcVI strain isolated in 1963 from naturally infected *Triatoma infestans* (kissing bug) collected from a house in Rio Grande do Sul, Brazil.[3-5]
Comment: *T. cruzi*, strain CL Brener (+*luc*) was deposited to BEI Resources as the epimastigote stage expressing the thermostable, red-shifted *Photinus pyralis* (North American firefly) luciferase gene mutant, *Ppy RE9*.[1,3,6] The complete genome of *T. cruzi*, strain CL Brener has been sequenced (GenBank: AAHK00000000).

The protozoan parasite *T. cruzi* is the causative agent of Chagas' disease, a debilitating vectorborne disease endemic in North, Central and South America.[7] In North America, *T. cruzi* has been identified through climactic and vector-based data as a potential emerging health risk to humans in the southern United States, where the two most commonly reported reservoirs in North America are the raccoon and the Virginia opossum.[8,9] The parasite has a complex life cycle and is transmitted by hematophagous triatomine reduviid bugs to wildlife and exotic mammal species, domestic dogs, and humans.[8,9] Dogs are considered a reservoir in the domestic transmission cycle of *T. cruzi* in endemic areas.[8,10]

T. cruzi is currently classified into six discrete typing units (DTUs; TcI, TcII, TcIII, TcIV, TcV and TcVI), which are identifiable by common molecular markers and represent different eco-epidemiological features, pathogenicity and geographical distribution.[3,11] TcVI is considered a hybrid DTU containing both TcII and TcIII genomes, and is most associated with domestic infection cycles of Chagas' disease in southern and central South America.[11]

The expression of red-shifted luciferase in *T. cruzi* results in a reporter system with increased sensitivity, allowing high-throughput screening of large numbers of compounds against the parasite *in vitro,* and has been used to track parasite distribution *in vivo* during long-term chronic infection of mice.[2,12]

Material Provided:

Each vial of NR-49161 contains approximately 0.5 mL of culture in cryopreservative. Please see Appendix I for cryopreservation instructions.

Packaging/Storage:

NR-49161 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:

Liver Infusion Tryptose (LIT) medium (ATCC® Medium 1029; Appendix II) adjusted to contain 10% (v/v) heat-inactivated fetal bovine serum
Incubation:
Temperature: 25°C
Atmosphere: Aerobic
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, transfer the vial contents to a T-25 tissue culture flask containing 10 mL of LIT medium. Incubate at 25°C with the cap screwed on tightly.
3. Observe the culture daily under an inverted microscope for the presence of epimastigote forms of the parasite. Subculture when the culture has reached peak density.

Maintenance:

1. Agitate a culture at or near peak density and aseptically transfer 0.5 mL to 1.0 mL into a new tissue culture flask with fresh growth medium.
2. Incubate the culture at 25°C with the cap screwed on tightly and examine daily under an inverted microscope.
3. Transfer every 3 to 7 days, as needed. Note that the transfer interval should be determined empirically as it is dependent on the quantity of the inoculum.

Please see Appendix I below for cryopreservation instructions.

Citation:

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH:

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Trypanosoma cruzi, Strain CL Brener (+*luc*), NR-49161."

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.

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APPENDIX I: CRYOPRESERVATION

1. To harvest the *Trypanosoma* culture, remove the media containing trypanosomes from infected culture flasks that have reached peak density and transfer to 15 mL plastic centrifuge tubes. Centrifuge at 800 × g for 10 min.
2. Remove all but 0.5 mL of the supernatant from each tube, resuspend the cell pellets, and pool them into a single tube.
3. Adjust the parasite concentration to 2.0×10^7 to 4.0×10^7 cells/mL using fresh growth medium.
   Note: If the concentration of parasites is too low, centrifuge at 800 × g for 10 min and resuspend in a smaller volume of fresh medium to yield the desired parasite concentration.
4. Mix equal volumes of parasite suspension and fresh medium containing 10% DMSO to yield a final concentration of 1.0×10^7 to 2.0×10^7 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 U/mL to 100 U/mL penicillin and 50 µg/mL to 100 µg/mL streptomycin.
5. Dispense 0.5 mL aliquots into 1 mL to 2 mL sterile plastic screw-capped vials for cryopreservation.
6. 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.
7. Store in either the vapor or liquid phase of a nitrogen refrigerator (-130°C or colder).

APPENDIX II: ATCC® Medium 1029

Component	Amount
Liver Infusion Broth (BD 226920)	9.0 g
Tryptose (BD 211713)	5.0 g
NaCl	1.0 g
Na_2HPO_4	8.0 g
KCl	0.4 g
Glucose	1.0 g
Fetal bovine serum (heat-inactivated)	100.0 mL
Hemin	10.0 mg
Distilled water to	1.0 L

Adjust pH to 7.2 and filter-sterilize. Dispense aseptically in 5.0 mL aliquots into 16 × 125 screw-capped test tubes.

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