Trypanosoma cruzi, Strain Tulahuen, Clone C4 (+*lacZ*)

Catalog No. NR-18959

For research use only. Not for use in humans.

Contributor:
F. S. Buckner, Associate Professor, Department of Medicine, University of Washington, Seattle, Washington, USA

Manufacturer:
BEI Resources

Product Description:
Protozoa Classification: *Trypanosomatidae*, *Trypanosoma*
Species: *Trypanosoma cruzi*
Strain: Tulahuen (clone C4; +*lacZ*)
Original Source: *Trypanosoma cruzi* (*T. cruzi*), strain Tulahuen, clone C4 (+*lacZ*) is a transgenic clone derived from the Tulahuen strain.[1] Strain Tulahuen is a highly pathogenic strain of *T. cruzi* that was originally isolated in Chile.[2]
Comment: *T. cruzi,* strain Tulahuen clone C4 (+*lacZ*) was deposited to BEI Resources as a β-galactosidase expressing strain consisting of the trypomastigote stage of the parasite's life cycle.

The protozoan parasite *T. cruzi* is the causative agent of Chagas' disease, a debilitating vectorborne disease endemic in North, Central and South America.[3] 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.[4,5] 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.[4,5] Dogs are considered a reservoir in the domestic transmission cycle of *T. cruzi* in endemic areas.[4,6]

The search for trypanosomicidal compounds can be facilitated by utilization of a colorimetric reaction to quantitate parasitic growth in tissue culture cells (modeling an *in vivo* infection). *T. cruzi,* strain Tulahuen clone C4 (+*lacZ*) has been genetically engineered to express *Escherichia coli* β-galactosidase (*lacZ*) an enzyme that can catalyze a colorimetric reaction with chlorophenol red β-D-galactopyranoside (CPRG).[1] The amount of enzymatic activity is directly proportional to the number of transfected parasites making this activity easily and accurately measured on a 96-well tissue culture plate using a microplate reader.[1] The utilization of the β-galactosidase expressing parasites facilitates high throughput screening of large numbers of candidate compounds against *T. cruzi*.[1]

Material Provided:
Each vial of NR-18959 contains approximately 0.5 mL of culture in cryopreservative [5% dimethylsulfoxide (DMSO)]. Please refer to Appendix I for cryopreservation instructions.

Packaging/Storage:
NR-18959 was packaged aseptically in 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 Modified Eagle's Medium (DMEM) (ATCC® 30-2002™) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (HIFBS)
BALB/3T3 clone A31 mouse embryonic fibroblast cells (ATCC® CCL-163™)
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 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 monolayer of BALB/3T3 clone A31 mouse embryonic fibroblasts 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 × g for 10 minutes.

3. Remove the supernatant and resuspend the cell pellet in a small volume (0.5 mL to 1 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.

Please refer to Appendix I for cryopreservation instructions.

Citation:

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: *Trypanosoma cruzi*, Strain Tulahuen, Clone C4 (+*lacZ*), NR-18959."

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 (BMBL). Current Edition. Washington, DC: U.S. Government Printing Office.

Disclaimers:

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References:

1. Buckner, F. S., et al. "Efficient Technique for Screening Drugs for Activity against *Trypanosoma cruzi* using Parasites Expressing β-Galactosidase." Antimicrob. Agents Chemother. 40 (1996): 2592-2597. PubMed: 8913471.
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3. Bern, C. "Chagas' Disease." N. Engl. J. Med. 373 (2015): 456-466. PubMed: 26222561.
4. Patel, J. M., et al. "Isolation, Mouse Pathogenicity, and Genotyping of *Trypanosoma cruzi* from an English Cocker Spaniel from Virginia, USA." Vet. Parasitol. 187 (2012): 394-398. PubMed: 22341614.
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6. Estrada-Franco, J. G., et al. "Human *Trypanosoma cruzi* Infection and Seropositivity in Dogs, Mexico." Emerg. Infect. Dis. 12 (2006): 624-630. PubMed: 16704811.
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BEI Resources
www.beiresources.org

E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898

APPENDIX I: CRYOPRESERVATION

1. To harvest the *Trypanosoma* culture, remove the media containing trypomastigotes from infected culture flasks that have reached peak density and transfer to 15 mL plastic centrifuge tubes. Centrifuge at 1300 × 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 2×10^7 to 4×10^7 cells/mL using fresh growth medium.
   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.
4. Mix equal volumes of parasite suspension and fresh medium containing 10% DMSO to yield a final concentration of 1×10^7 to 2×10^7 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).

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E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898