Trypanosoma cruzi, Strain TcVT-1 (axenic epimastigote)

Catalog No. NR-46428

For research use only. Not for human use.

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Manufacturer:
BEI Resources

Product Description:
Protozoa Classification: *Trypanosomatidae*, *Trypanosoma*
Species: *Trypanosoma cruzi*
Strain: TcVT-1 (axenic epimastigote)
Original Source: *Trypanosoma cruzi (T. cruzi)*, strain TcVT-1 was isolated from the blood of a five-year-old female English Cocker Spaniel with Chagas' disease in October 2012 in Lynchburg, Virginia, USA.[1,2]
Comment: *T. cruzi,* strain TcVT-1 was deposited as the trypomastigote form of the parasite's life cycle and a genotype TcIV strain.[1,2] NR-46428 consists of the epimastigote stage, which was established by BEI Resources from the trypomastigote form over multiple passages in culture.

The protozoan parasite *T. cruzi* is the causative agent of Chagas' disease, a debilitating disease endemic in many Latin American countries. 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.[1,3] 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.[1,3] Dogs are considered a reservoir in the domestic transmission cycle of *T. cruzi* in endemic areas.[1,4]

Material Provided:
Each vial of NR-46428 contains approximately 0.5 mL of culture in cryopreservative [5% dimethylsulfoxide (DMSO)]. Please refer to the Certificate of Analysis for the specific culture media used for each lot and refer to Appendix I for cryopreservation instructions.

Packaging/Storage:
NR-46428 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 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) adjusted to contain 10% (v/v) heat-inactivated fetal bovine serum and 1% hemin (Appendix II)
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 bloodstream 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 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 TcVT-1 (axenic epimastigote), NR-46428."

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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References:

1. Lindsay, D. S., Personal Communication.
2. 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.
3. Brown, E. L., et al. “Seroprevalence of *Trypanosoma cruzi* among Eleven Potential Reservoir Species from Six States across the Southern United States.” Vector Borne Zoonotic Dis. 10 (2010): 757-763. PubMed: 20020815.
4. 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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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 × 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 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: LIVER INFUSION TRYPTOSE (LIT) MEDIUM (ATCC® MEDIUM 1029)

1. Prepare the LIT base medium using the formula listed below:

Liver Infusion Broth Dehydrated Powder (BD Difco™ 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

2. Bring the final volume up to 1 L with distilled water.
3. Adjust pH to 7.2 and filter sterilize using a 0.2 µm filter.
4. Aseptically supplement the LIT base medium with 10% heat-inactivated fetal bovine serum and 1% hemin.

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