Trypanosoma cruzi, Strain Dm28c

Catalog No. NR-49380

For research use only. Not for use in humans.

Contributor:

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Manufacturer:

BEI Resources

Product Description:

Protozoa Classification: *Trypanosomatidae*, *Trypanosoma*
Species: *Trypanosoma cruzi*
Strain: Dm28c
Original Source: *Trypanosoma cruzi (T. cruzi)*, strain Dm28c is a clone derived from *T. cruzi,* strain Dm28.[1,2,3] Strain Dm28 was isolated in 1984 from an opossum (*Didelphis marsupialis)* in Carabobo, Venezuela.[1,2,3]
Comments: *T. cruzi*, strain Dm28c corresponds to discrete typing unit (DTU) *T. cruzi* I (TcI) based on sequence analysis of the putative C-5 sterol desaturase gene, *TcSC5D*.[1,4] *T. cruzi*, strain Dm28c was deposited to BEI Resources as the epimastigote stage of the parasite's life cycle.[2] The complete genome for *T. cruzi*, strain Dm28c has been sequenced (GenBank: AYLP00000000).[4]

The protozoan parasite *T. cruzi* is the causative agent of Chagas' disease, a debilitating vectorborne disease endemic in North, Central and South America.[5] 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.[6,7] 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.[6,7] Dogs are considered a reservoir in the domestic transmission cycle of *T. cruzi* in endemic areas.[6,8]

T. cruzi is currently classified into six discrete typing units (TcI, TcII, TcIII, TcIV, TcV and TcVI), which are identifiable by common molecular markers and represent different eco-epidemiological features, pathogenicity and geographical distribution.[1,9] TcI is the most abundant and widespread DTU among the Americas, with vector distribution in both sylvatic and domestic infection cycles.[9]

Material Provided:

Each vial of NR-49380 contains approximately 0.5 mL of culture in cryopreservative [5% dimethylsulfoxide (DMSO)]. Please refer to Appendix I for cryopreservation instructions.

Packaging/Storage:

NR-49380 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:

Liver Infusion Tryptose (LIT) medium (Appendix II) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (HIFBS)
Incubation:
Temperature: 37°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, aseptically transfer the contents to a 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 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 Dm28c, NR-49380."

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). 6th ed. Washington, DC: U.S. Government Printing Office, 2020.

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References:

1. Zingales, B., et al. "A New Consensus for *Trypanosoma cruzi* Intraspecific Nomenclature: Second Revision Meeting Recommends TcI to TcVI." Mem. Inst. Oswaldo Cruz 104 (2009): 1051-1054. PubMed: 20027478.
2. Buck, G., Personal Communication.
3. Goldenberg, S., et al. "Facts and Hypothesis on *Trypanosoma cruzi* Differentiation." Mem. Inst. Oswaldo Cruz 79 (1984): 39-44.
4. Grisard, E. C., et al. "*Trypanosoma cruzi* Clone Dm28c Draft Genome Sequence." Genome Announc. 2 (2014): e01114-13. PubMed: 24482508.
5. Bern, C. "Chagas' Disease." N. Engl. J. Med. 373 (2015): 456-466. PubMed: 26222561.
6. 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.
7. 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.
8. Estrada-Franco, J. G., et al. "Human *Trypanosoma cruzi* Infection and Seropositivity in Dogs, Mexico." Emerg. Infect. Dis. 12 (2006): 624-630. PubMed: 16704811.
9. Zingales, B., et al. "The Revised *Trypansoma cruzi* Subspecific Nomenclature: Rationale, Epidemiological Relevance and Research Applications." Infect. Genet. Evol. 12 (2012): 240-253. PubMed: 22226704.

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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 2×10^7 to 4×10^7 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 1×10^7 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).

APPENDIX II: LIVER INFUSION TRYPTOSE (LIT) MEDIUM

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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