



Trypanosoma cruzi Chagas

50832_TT™

Description

Strain designation: Y

Deposited As: *Trypanosoma cruzi* Chagas

Type strain: No

Storage Conditions

Product format: Test tube

Storage conditions: See handling procedure

Intended Use

This product is intended for laboratory research use only. It is not intended for any animal or human therapeutic use, any human or animal consumption, or any diagnostic use.

BSL 2

ATCC determines the biosafety level of a material based on our risk assessment as guided by the current edition of *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, U.S. Department of Health and Human Services. It is your responsibility to understand the hazards associated with the material per your organization's policies and procedures as well as any other applicable regulations as enforced by your local or national agencies.

ATCC highly recommends that appropriate personal protective equipment is always used when handling vials. For cultures that require storage in liquid nitrogen, it is important to note that some vials may leak when submerged in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vial exploding or blowing off its cap with dangerous force creating flying debris. Unless necessary, ATCC recommends that these cultures be stored in the vapor phase of liquid nitrogen rather than submerged in liquid nitrogen.

Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at www.atcc.org.

Growth Conditions

Medium:

ATCC Medium 1029: LIT medium

Temperature: 25°C

Culture system: Axenic

Handling Procedures

Handling of Live Culture

Do not store the culture at refrigeration temperatures before handling. To assure viability, immediately place the culture on a 15-degree slant from horizontal and incubate with cap tight at 20-25°C for at least one hour before observing the culture. There should be numerous active trophozoites (epimastigotes) in suspension

and/or settled along the side of the tube facing down. If the numbers are low the culture may have been exposed to temperature extremes in transit. Regardless of the state of the culture, mix well and aseptically transfer 0.5 mL and 1.0 mL aliquots to a pair of T-25 tissue culture flasks containing 10 mL fresh, complete medium each. If little or no movement is seen in the parent culture tube, that culture may optionally be re-fed by transferring the entire contents to a 15 mL plastic centrifuge tube and centrifuging at $\sim 1000 \times g$ for 6-8 min. After centrifugation, remove all but ~ 1 mL of the supernatant and then resuspend pelleted cells in ~ 5 mL fresh, complete medium. Incubate all cultures with caps tightened at 20-25°C (test tubes should be incubated on a 15-degree slant from horizontal). Thereafter follow the protocol for maintenance of culture.

Culture maintenance:

1. Agitate a culture at or near peak density and aseptically transfer 0.1-0.2 mL to a fresh flask of ATCC medium 1029.
2. Incubate at 20-25°C with the cap screwed on tightly.
3. Transfer the culture every 14-21 days as described in steps 1-2. The transfer interval will depend on the quantity of the inoculum and the quality of the medium. This should be empirically determined by examining the culture on a daily basis until the growth cycle has stabilized.

Cryopreservation:

1. Harvest cells from a culture that is at or near peak density by centrifugation at $\sim 800 \times g$ for 5 min. Pool the cell pellets into a single tube.
2. Adjust the concentration of cells to 2.0×10^7 /mL. If the concentration is too low, centrifuge at $\sim 800 \times g$ for 5 minutes and resuspend the cell pellet with a volume of supernatant to yield the desired concentration.
3. Prepare a 10% (v/v) sterile DMSO solution in fresh medium as follows: Add the required volume of DMSO to a glass screw-capped test tube and place on ice. Allow the DMSO to solidify. Add the required volume of refrigerated medium. Dissolve the DMSO by inverting several times. If the DMSO solution is not prepared on ice, an exothermic reaction will occur that may precipitate certain components of the medium.
4. Mix the cell preparation and the DMSO in equal portions. Thus, the final concentration will be 10^7 and 5% (v/v) DMSO. The time from the mixing of the cell preparation and DMSO stock solution to the start of the freezing process should be no less than 15 min and no longer than 60 min.
5. Dispense in 0.5 mL aliquots into 1.0 - 2.0 mL sterile plastic screw-capped

- cryules (special plastic vials for cryopreservation).
6. Place vials in a controlled rate freezing unit. From room temperature cool at -1°C/min to -40°C. If freezing unit can compensate for the heat of fusion, maintain rate at -1 C/min through heat of fusion. At -40°C plunge ampules into liquid nitrogen.
 7. The frozen preparations are stored in either the vapor or liquid phase of a nitrogen refrigerator.
 8. To establish a culture from the frozen state place an ampule in a water bath set at 35°C. Immerse the vial enough to cover only the frozen material. Do not agitate the vial.
 9. Immediately after thawing, do not leave in the water bath, aseptically remove the contents of the ampule and inoculate into 10.0 mL of fresh ATCC medium 1029.
 10. Incubate the tube at 20-25°C with the cap screwed on tightly.
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Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Trypanosoma cruzi* Chagas (ATCC 50832_TT)

References

References and other information relating to this material are available at www.atcc.org.

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Revision

This information on this document was last updated on 2022-10-22

Contact Information

ATCC

10801 University Boulevard

Manassas, VA 20110-2209

USA

US telephone: 800-638-6597

Worldwide telephone: +1-703-365-2700

Email: tech@atcc.org or contact your local distributor
