

30013[™]

Description

Strain designation: Culbertson

Deposited As: Trypanosoma cruzi Chagas

Type strain: No

Storage Conditions

Product format: Frozen

Storage conditions: -80°C or colder for 1 week, vapor phase of liquid nitrogen for

long-term storage

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.

BSL₂

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 submersed 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 submersed 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

ATCC Medium 1012: Diphasic blood agar medium

Instructions for complete medium: Media: 1029 LIT medium.

Alternate Media: 1012 diphasic blood agar medium can be used

Temperature: 25°C **Culture system:** Axenic

Handling Procedures

Storage and Culture Initiation

Frozen ampules packed in dry ice should either be thawed immediately or stored in

liquid nitrogen. If liquid nitrogen storage facilities are not available, frozen ampules may be stored at or below -70°C for approximately one week. **Do not under any circumstance store frozen ampules at refrigerator freezer temperatures (generally -20°C).** Storage of frozen material at this temperature will result in the death of the culture.

- 1. To thaw a frozen ampule, place it in a 35°C water bath , until thawed (2-3 min). Immerse the ampule just sufficient to cover the frozen material. Do not agitate the ampule.
- 2. Immediately after thawing, aseptically transfer contents to a T-25 tissue culture flask containing 10 mL of ATCC Medium 1029. Incubate at 25°C.

Culture maintenance:

- 1. Agitate a culture at or near peak density by inverting several times.
- 2. Aseptically transfer a 0.1 mL aliquot to a T-25 tissue culture flask containing 10 mL of fresh complete medium.
- 3. Screw cap on tightly and incubate at 20°C to 25°C.
- 4. Subculture every 14-21d.

Cryopreservation:

- 1. Harvest cells from several cultures in very late logarithmic to early stationary phase of growth. Vigorously agitate to suspend the cells.
- 2. Aseptically transfer the cell suspension to 15 mL plastic centrifuge tubes.
- 3. Centrifuge at ~800 x g for 5 min.
- 4. While cells are centrifuging, prepare a 10% solution of DMSO in complete ATCC Medium 1029. Cool on ice.
- 5. Remove the supernatant and pool the cell pellets to the final volume desired with fresh growth medium.
- 6. Combine the cell suspension with an equal volume of 10% DMSO cryoprotectant solution (prepared in step 4) to yield a final concentration of 5% DMSO.
- 7. Dispense in 0.5 mL aliquots to 1.0-2.0 mL Nunc vials (special plastic vials for cryopreservation).
- 8. Place the vials in a controlled rate freezing unit. From room temperature cool at -1°C/min to -40°C. At -40°C, plunge ampules into liquid nitrogen.

 Alternatively, place the vials in a Nalgene 1°C freezing apparatus. Place the apparatus at -80°C for 1.5 to 2 hours and then plunge ampules into liquid nitrogen. (The cooling rate in this apparatus is approximately -1°C/min.).
- 9. Store ampules in a liquid nitrogen refrigerator until needed.
- 10. To establish a culture from the frozen state, place a frozen ampule in a 35°C



- water bath just enough to cover the frozen material. Allow the ampule to thaw completely (2-3 min).
- 11. Immediately after thawing, aseptically remove the contents and transfer to a T-25 tissue culture flask containing 10 mL of fresh complete ATCC medium 1029.
- 12. Screw the cap on tightly and incubate at 20°C to 25°C. Observe the culture daily and transfer when numerous trophozoites are observed.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Trypanosoma cruzi* Chagas (ATCC 30013)

References

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

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