Trypanosoma cruzi Chagas

PRA-333™

Description

Strain designation: VL2067 LacZ clone A8
Type strain: No

Storage Conditions

Product format: Frozen

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 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

Host: BALB/3T3 clone A31 (ATCC CCL-163)
Medium:
ATCC Medium 2222: Cell Cultivation Medium for Parasites
Temperature: 35-37°C

Handling Procedures

Cryopreservation:

1. Harvest *Trypanosoma* cultures when emergent parasites (trypomastigote stage) have reached or are near peak density in the liquid column. Gently invert the *Trypanosoma* culture flasks to suspend parasites in the liquid medium.

2. Transfer the cell suspension (including parasites) to 15 ml plastic centrifuge tubes. Centrifuge at 1300 x g for 10 min.

3. Remove all but 0.5 ml of the supernatant from each tube, resuspend the cell pellets, and pool them to a single tube.

4. Adjust the parasite concentration to 2.0 - 4.0 x 10^7 cells/ml with fresh medium or PBS.

NOTE: If the concentration of parasites is too low, centrifuge at 1300 x g for 10 min and resuspend in the volume of fresh medium or PBS required to yield the desired concentration.

5. Prepare a cryoprotective solution containing 10% (v/v) DMSO in fresh medium or PBS.

6. Mix the cell preparation and cryoprotective solution in equal portions. The final concentration will be 1.0 - 2.0 x 10^7 cells/ml and 5% DMSO. The time from the mixing of the cell preparation and cryoprotective solution to the start of the freezing process should be no less than 15 min and no more than 30 min.

NOTE: To prevent culture contamination, penicillin-streptomycin solution (ATCC® 30-2300) may be added to a final concentration of 50 to 100 I.U./ml penicillin and 50 to 100 µg/ml streptomycin.

7. Dispense in 0.5 ml aliquots to 1.0-2.0 ml sterile plastic screw-capped cryovials.

8. Place cryovials 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. 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 frozen ampules in either the vapor or liquid phase of a nitrogen refrigerator.

10. To thaw a frozen ampule, place it in a 35-37°C water bath such that the lip of the ampule remains above the water line. Thawing time is approximately 2 to 3 minutes. Do not agitate the ampule. Do not leave ampule in water bath after thawed.

11. Immediately after thawing, aseptically transfer contents to a T-25 tissue culture flask containing a fresh monolayer of ATCC® CCL-163 cells and 10 ml ATCC®

30-2002 with 10% (v/v) HIFBS.

12. Outgas the flask for 10 seconds with a 95% air, 5% CO_2 gas mixture.

13. Incubate in a 35-37°C CO_2 incubator with the cap screwed on tightly.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Trypanosoma cruzi* Chagas (ATCC PRA-333)

References

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

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