ATCC

Product Sheet

Trypanosoma brucei rhodesiense (ATCC® 30027™)

Please read this FIRST

Storage Temp.
Frozen Cultures:
-70°C for 1 week; liquid N_2 vapor for long term storage

Freeze-dried Cultures:
2-8°C

Live Cultures:
See Protocols section for handling information

Biosafety Level
2

Intended Use

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

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: *Trypanosoma brucei rhodesiense* (ATCC® 30027™)

American Type Culture Collection
PO Box 1549
Manassas, VA 20108 USA
www.atcc.org

800.638.6597 or 703.365.2700
Fax: 703.365.2750
Email: Tech@atcc.org

Or contact your local distributor

Description

Strain Designation: Wellcome CT
Deposited Name: *Trypanosoma rhodesiense* Stephens and Fantham
Depositor: EJ Tobie
Isolation: Human, Tinde, Tanganyika, 1934

Propagation

Growth Conditions
Culture System: *in vivo*, laboratory rat

Protocols

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 ampoules 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 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 it is thawed.
2. Immediately after thawing, aseptically remove the entire contents of the ampule with a syringe and inject intraperitoneally into an adult rat known to be free of blood parasites. Follow the protocol for maintenance *in vivo*. The course of infection may be longer or shorter than usual depending on percent recovery of the parasite from the frozen state.

Culture Maintenance

1. Monitor the course of infection by drawing a drop of blood from the tail of an infected animal, mixing with 1.0 mL of buffered saline (0.8% saline buffered to pH 7), and observing microscopically. If one or more parasites are seen, follow the procedures in step 2.
2. To infect additional rats, draw a drop of blood from the tail vein of an infected animal into a syringe containing 0.5 mL of sterile buffered anticoagulant, mix, and inoculate 0.1-0.2 mL of the suspension intraperitoneally into an adult rat known to be free of blood parasites.
3. Parasites for inoculation should be collected at or near peak parasitemia (usually within 4-8 days of infection if large inocula are given). Peak parasitemia from the thawed material may be slightly sooner or later. Host rats should be approximately 4-6 months old.

Cryopreservation

Reagents
Tyrode's Salt Solution
NaCl, 8.00 g
KCl, 0.20 g
$CaCl_2$, 0.20 g
$MgCl_2 \cdot H_2O$, 0.05 g
$NaH_2PO_4 \cdot H_2O$, 1.00 g
$NaHCO_3 \cdot H_2O$, 1.00 g
Glucose, 1.00 g
Glass distilled H_2O to 1.00 L
Add ingredients in the sequence listed. Filter-sterilize.

Harvest and Preservation

1. Harvest the parasites according to the protocol for maintenance *in vivo*.
2. Spin the cell suspension at approximately 50 x g for 3 min, to remove any cellular debris.
3. Transfer the supernatant to a new 15 mL plastic centrifuge tube. Centrifuge at 1300 x g for 10 min.
4. Pool the cell pellets and adjust the concentration to 2.0 - 4.0 x 10^7 cells/mL with a fresh solution of Tyrode's Salt Solution. *If the concentration is too low centrifuge at 1300 x g for 10 min and resuspend in the volume of Tyrode's Salt Solution required to yield the desired concentration.
5. Mix the cell preparation and 10% (v/v) DMSO in equal portions. The final concentration will be 1.0 -

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2.0×10^7 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.
6. Dispense in 0.5 mL aliquots to 1.0-2.0 mL sterile plastic screw-capped cryules (special plastic vials for cryopreservation).
7. Place the vials in a controlled rate freezing unit. From room temperature cool 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 the heat of fusion. At -40°C plunge 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.)
8. Store in either the vapor or liquid phase of a nitrogen refrigerator.
9. To thaw a frozen ampule, place it in a 35°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.
10. Immediately after thawing, aseptically remove the contents of the ampule with a syringe and inoculate an uninfected rat. Follow the protocol for maintenance *in vivo*.

References

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

Biosafety Level: 2

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the current publication of the *Biosafety in Microbiological and Biomedical Laboratories* from the U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes for Health.

ATCC Warranty

The viability of ATCC® products is warranted for 30 days from the date of shipment, and is valid only if the product is stored and cultured according to the information included on this product information sheet. ATCC lists the media formulation that has been found to be effective for this strain. While other, unspecified media may also produce satisfactory results, a change in media or the absence of an additive from the ATCC recommended media may affect recovery, growth and/or function of this strain. If an alternative medium formulation is used, the ATCC warranty for viability is no longer valid.

Disclaimers

This product is intended for laboratory research purposes only. It is not intended for use in humans.

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Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at www.atcc.org

Additional information on this culture is available on the ATCC web site at www.atcc.org.