Leishmania donovani (Laveran and Mesnil) Ross

30498™

Description

Strain designation: 1S
Deposited As: *Leishmania donovani* (Laveran and Mesnil) Ross
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:

in vivo cultivation, golden hamster

Temperature: 25°C

Handling Procedures

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 uninfected Golden hamster at least 8 weeks old. 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. Inoculate entire infected blood suspension intraperitoneally into the hamster using a 1.0 mL syringe equipped with a 27 gauge 1/2 inch needle. Serial passage after 30-45 days.
2. To infect additional hamsters, harvest the parasites from spleen tissue homogenized in a balanced salt solution (i.e., Tyrodes' Salt solution or similar), approximately 5.0 mL salt solution per spleen. Inoculate 0.3-0.5 mL of spleen homogenate per hamster. Dosage should be a minimum of 2 x 106 amastigotes per hamster. The course of infection will vary with the size of the inoculum.
3. Parasites for inoculation should be collected within 30-45 days of infection. Host hamsters should be at least 8 weeks old.

Reagents for cryopreservation: Tyrode's Salt Solution

NaCl 8.00 g
KCl 0.20 g
$CaCl_2$ 0.20 g
$MgCl_2*H_2O$ 0.05 g
$NaH_2PO_4*H_2O$ 1.00 g
$NaHCO_3*H_2O$ 1.00 g
Glucose 1.00 g
Glass distilled H_2O to 1.00 L

Add ingredients in the sequence listed. Filter-sterilize.

1. **Cryopreservation:**
2. Harvest the parasites from spleen tissue homogenized in a balanced salt solution (i.e., Tyrodes' Salt soln. or similar), approximately 5.0 mL solution per spleen.
3. Transfer the cell homogenate to a 15 mL plastic centrifuge tube and spin at approximately 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 - 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.
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 Golden hamster at least 8 weeks old. Follow the protocol for maintenance *in vivo*.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Leishmania donovani* (Laveran and Mesnil) Ross (ATCC 30498)

References

References and other information relating to this material are available at

www.atcc.org.

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