

PRA-409[™]

Description

Strain designation: Lab 110 EATRO

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

Host:

in vivo cultivation, Balb/c mouse

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 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

PRA-409

- 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 contents of the ampule with a syringe and inoculate a Balb/c mouse. Follow the protocol for maintenance of the culture below. The course of infection may depend on the parasite strain and recovery from the frozen state.

Culture maintenance: Reagents

Yaeger's anticoagulant
Sodium citrate, 1.33 g
Citric acid, 0.47 g
Dextrose, 3.00 g
Sodium heparin, 0.20 g
Glass distilled H₂O to 100.00 mL

- 1. Inoculate entire infected blood suspension intraperitoneally into a Balb/c mouse using a 1.0 mL syringe equipped with a 27 gauge 1/2 inch needle.
- 2. Bleed the mouse at daily intervals to monitor parasitemia by microscopic examination using a haemocytometer and 0.85% ammonium chloride as diluent. Parasitemia may also be assessed by microscopic examination of blood films stained with 5% Giemsa solution.
- 3. Passage the strain when the infection is at a parasitemia of $\ge 5 \times 10^5$ parasites/mL or ≥ 5 parasites/HPF for Giemsa-stained blood films observed under 100X. This will normally occur after 2-3 days of inoculation. Note that the rate of *T. brucei brucei* infection may vary with the parasite strain.
- 4. To passage the strain, anesthetize the first infected mouse by CO_2/O_2 inhalation. Collect the blood by orbital bleeding or from the tail vein using an anticoagulant such as Yaeger's anticoagulant solution or EDTA.
- 5. Perform a parasite count and inject 5×10^4 to 1×10^5 parasites into a determined number of Balb/c mice (~10).
- 6. Monitor parasitemia as described above and passage as needed.

NOTE: Cardiac puncture may be used as an alternative method of blood collection.

Reagents for cryopreservation: Trypanosome Dilution Buffer

20 mM Na₂HPO₄ 2 mM NaH₂PO₄ 80 mM NaCl



PRA-409

5 mM KCl 1 mM MgSO₄ 20 mM glucose

Adjust the pH of the solution to 7.7 and filter sterilize.

Cryopreservation: br /> Harvest and Preservation

- 1. Prepare a 40% (v/v) sterile glycerol solution in Trypanosome Dilution Buffer (TDB).
- 2. Dispense 0.5 mL of anticoagulant solution into a 15 mL test tube. Add to the anticoagulant blood collected by orbital bleeding from mice that had reached or are near peak parasitemia. Invert the tube several times to mix the blood with the anticoagulant.
- 3. In a separate test tube, add the heparinized blood dropwise to the 40% glycerol solution. Note that blood should be mixed with glycerol solution in a 1:1 ratio to obtain a final concentration of cryoprotectant of 20% (v/v). Mix slowly by inversion and place the tube on ice. The freezing process should start 15 to 30 minutes following the addition of the heparinized blood to the cryoprotectant solution.
- 4. Dispense 0.5 mL aliquots of blood suspension into 1.0 2.0 mL sterile plastic screw-capped cryovials. Place the vials in a controlled rate freezing unit. From room temperature cool the vials 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 this phase. At -40°C, plunge vials into liquid nitrogen. Alternatively, place the vials in a Nalgene 1°C freezing container. Place the container at -80°C for 1.5 to 2 hours and then plunge vials into liquid nitrogen.
- 5. To thaw a frozen ampule, place 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.
- 6. Immediately after thawing, aseptically remove the contents of the ampule with a syringe and inoculate a Balb/c mouse. Follow the protocol for maintenance of the culture above.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Trypanosoma brucei brucei* (ATCC PRA-409)

PRA-409

References

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

Warranty

The product is provided 'AS IS' and the viability of ATCC® products is warranted for 30 days from the date of shipment, provided that the customer has stored and handled the product according to the information included on the product information sheet, website, and Certificate of Analysis. For living cultures, ATCC lists the media formulation and reagents that have been found to be effective for the product. While other unspecified media and reagents may also produce satisfactory results, a change in the ATCC and/or depositor-recommended protocols may affect the recovery, growth, and/or function of the product. If an alternative medium formulation or reagent is used, the ATCC warranty for viability is no longer valid. Except as expressly set forth herein, no other warranties of any kind are provided, express or implied, including, but not limited to, any implied warranties of merchantability, fitness for a particular purpose, manufacture according to cGMP standards, typicality, safety, accuracy, and/or noninfringement.

Disclaimers

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. Any proposed commercial use is prohibited without a license from ATCC.

While ATCC uses reasonable efforts to include accurate and up-to-date information on this product sheet, ATCC makes no warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. ATCC does not warrant that such information has been confirmed to be accurate or complete and the customer bears the sole responsibility

PRA-409

of confirming the accuracy and completeness of any such information.

This product is sent on the condition that the customer is responsible for and assumes all risk and responsibility in connection with the receipt, handling, storage, disposal, and use of the ATCC product including without limitation taking all appropriate safety and handling precautions to minimize health or environmental risk. As a condition of receiving the material, the customer agrees that any activity undertaken with the ATCC product and any progeny or modifications will be conducted in compliance with all applicable laws, regulations, and guidelines. This product is provided 'AS IS' with no representations or warranties whatsoever except as expressly set forth herein and in no event shall ATCC, its parents, subsidiaries, directors, officers, agents, employees, assigns, successors, and affiliates be liable for indirect, special, incidental, or consequential damages of any kind in connection with or arising out of the customer's use of the product. While reasonable effort is made to ensure authenticity and reliability of materials on deposit, ATCC is not liable for damages arising from the misidentification or misrepresentation of such materials.

Please see the material transfer agreement (MTA) for further details regarding the use of this product. The MTA is available at www.atcc.org.

Copyright and Trademark Information

© ATCC 2023. All rights reserved.

ATCC is a registered trademark of the American Type Culture Collection.

Revision

This information on this document was last updated on 2024-10-26

Contact Information

ATCC

10801 University Boulevard



PRA-409

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

