



***Babesia microti* (Franca) Reichenow**

30221™

Product Sheet

Description

Babesia microti strain Gray is a parasitic protozoan that was isolated from a male with piroplasms from Nantucket Island, Massachusetts. This strain is cultivated in hamsters and can be used in infectious and vector-borne disease research.

Strain designation: Gray

Deposited As: *Babesia microti* (Franca) Reichenow

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.

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

30221

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, hamster

Handling Procedures

Storage and Culture Initiation

NOTE: See the protocol for maintenance *in vivo* in the next section prior to thawing the material.

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

30221

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 ampoules 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 contents of the ampule with a syringe and inoculate an uninfected hamster. 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: 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

NOTE: Immunosuppression of the hamster host is recommended in order to obtain higher levels of parasitemia.

1. Immunosuppress a hamster by intraperitoneal injection of cortisone (2mg/day/hamster) or cyclophosphamide (100mg/kg) 1-3 days prior to inoculation.
2. Inoculate entire infected blood suspension intraperitoneally into a hamster using a 1.0 mL syringe equipped with a 27 gauge 1/2 inch needle.
3. Monitor the infection daily or at 2 day intervals by examination of blood films stained with 5% Giemsa solution.
4. Count the number of infected red blood cells (rbc) versus the total number of red cells under oil immersion and determine the % parasitemia: % parasitemia = infected rbc / rbc X 100 A minimum of 500 red blood cells should be counted. (Note that a red blood cell infected with multiple parasites is counted as a single infected cell.)
5. When the level of parasitemia is ≥ 10% the strain should be passaged. Normally this would occur 1-3 weeks post-inoculation, but the rate of infection may vary

30221

considerably. (Note that the level of parasitemia before the host will succumb will vary with the strain used. Monitoring on a daily basis will alert the experimenter as to when the strain should be passaged.)

6. To passage the strain, remove blood from the infected hamster using cardiac puncture using a syringe and suitable anticoagulant:
 - a. In a laminar flow hood ventilated to the outside, add one capful of the Metofane (Pitman-Moore, Inc. Washington Cross, NJ, cat# 55685) to a wad of cotton at the bottom of a gallon jar. Place a wire mesh screen over the top of the cotton and tightly secure the lid. Allow the jar to remain undisturbed for 10 minutes. Remove the lid of the jar and add the infected hamster. When the animal is thoroughly anesthetized, tie it down firmly with its stomach upward. Thoroughly swab the chest with 70% denatured alcohol.
 - b. Add 0.5 mL of anticoagulant solution (Yaeger's or heparin, etc.) to a 5.0 mL syringe equipped with a 27 gauge 1/2 inch needle. Puncture the heart and move the plunger of the syringe back and forth several times to distribute the anticoagulant.
 - c. Draw blood into the syringe by gently pulling the plunger outward. When blood is no longer obtainable or the hamster has died, remove the needle from the animal and invert the syringe several times to mix the anticoagulant evenly with the blood.
 - d. Remove air bubbles from the syringe. Place the syringe in a vertical position with the needle pointing upward. Place the tip of the needle on the surface of a thoroughly alcoholized cotton ball (squeeze the cotton ball so that it is moist but not dripping wet). With the index finger flick the top of the syringe several times to allow the air bubbles to coalesce and move to the top of the syringe body. Gently push in the plunger to remove the air pocket. It may be necessary to repeat this procedure several times to remove all the air bubbles. When a steady stream of blood exits the needle, the blood is ready for injection.
7. Inject 0.5 mL of the infected blood suspension into each uninfected hamster.
8. Monitor parasitemia and passage as needed.

Reagents for cryopreservation: Alsever's Solution

NaCl, 4.2 g

Na₃citrate·2H₂O, 8.0 g

Glucose, 20.5 g

Glass distilled H₂O to 1.0 L

30221

*Dissolve components in glass distilled H₂O, adjust the pH to 6.1 with 10% (w/v) citric acid and filter sterilize. The solution can be obtained from Sigma-Aldrich (cat# A3551).

Cryopreservation:

1. Prepare a 30% (v/v) sterile glycerol solution in Alsever's solution.
2. Draw approximately 0.5 mL of anticoagulant solution (Yaeger's or heparin, etc.) into a syringe and move it back and forth over the length of the syringe, several times. Remove all air bubbles. Draw blood by cardiac puncture into the syringe from a host animal that has reached or is near peak parasitemia. If clotting occurs during extraction of blood, insufficient heparin was used.
3. Mix the heparinized blood with the 30% glycerol solution in a 2:1 ratio. If any clotting has occurred do not use. After mixing, the final concentration of cryoprotectant solution will be 10% (v/v). The mixture should be placed in a 4°C ice bath. The time from the mixing of the cell preparation and glycerol stock solution before the freezing process is begun should be no less than 15 min and no longer than 30 min.
4. Dispense in 0.5 mL aliquots into 1.0 - 2.0 mL sterile plastic screw-capped cryules (special plastic vials for cryopreservation). Filled ampules should be placed in a 4°C ice bath. Do not immerse ampules to the level of the vial cap.
5. Plunge ampules from 4°C into liquid nitrogen. The frozen preparations may be stored in a mechanical freezer until needed, however storage in either the vapor or liquid phase of a nitrogen refrigerator is recommended for the longest viability.
6. 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.
7. Immediately after thawing, aseptically remove the contents of the ampule with a syringe and inoculate an uninfected hamster. 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.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Babesia microti* (Franca) Reichenow (ATCC 30221)

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

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 2022-10-22

Contact Information

ATCC

10801 University Boulevard

***Babesia microti* (Franca) Reichenow**

30221

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
