Babesia microti (Franca) Reichenow

PRA-398™

Description

Babesia microti strain GI (Ingram strain) is a parasitic protozoan that was isolated in 1983 in Nantucket, Massachusetts, from the blood of a human with babesiosis. This strain requires in vivo cultivation in a Golden Syrian hamster.

Strain designation: GI (Ingram strain)

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 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 Syrian hamster

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 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 of the culture below. The course of infection may be longer or shorter than usual depending on 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 H_2O to: 100.00 mL

1. Inoculate up to 0.5 mL of infected blood intraperitoneally into a hamster using facility approved methods.
2. Monitor the infection daily or at 2-day intervals by examination of blood films stained with 5% Giemsa solution.
3. 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.)
4. 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 considerably. (Note that the level of parasitemia before the host will succumb will vary with the strain used. Monitoring parasitemia as described above will alert the experimenter as to when the strain should be passaged.)
5. To passage the strain, collect blood from the infected hamster using cardiac puncture or other facility approved method using a syringe and suitable anticoagulant.
6. Anesthetize the animal by a facility approved method for anesthesia. Collect blood by cardiac puncture or other facility approved blood collection method.

7. Add approximately 0.05 - 0.1 mL of anticoagulant solution (Yaeger's or heparin, etc.) to a syringe.
8. Transfer blood to a collection tube containing and additional 0.05 – 0.1 mL anticoagulant per mL of anticipated blood collection. Mix the blood and anticoagulant by gentle repeated inversion of the tube to prevent clotting of the blood.
9. If necessary, blood may be diluted with Alsever's solution.
10. Inject up to 0.5 mL of the infected blood suspension into uninfected hamster(s) to passage or expand the infection as needed. Monitor parasitemia and passage as needed.

Reagents for cryopreservation: Alsever's Solution

NaCl, 4.2 g

Na_3citrate·$2H_2O$, 8.0 g

Glucose, 20.5 g

Glass distilled H_2O to 1.0 L

*Dissolve components in glass distilled H_2O, 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 0.05 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 or other facility approved blood collection method from a host animal that has reached desired parasitemia. Transfer blood to a collection tube containing additional anticoagulant (0.05 – 0.1 mL per mL of anticipated blood collection). If clotting occurs during extraction of blood, insufficient heparin was used. Mix the heparinized blood with the 30% glycerol solution in a 2:1 ratio to obtain a final concentration of cryoprotectant of 10% (v/v). Mix slowly by inversion and place the mixture on ice. The freezing process should start 15 to 30 minutes following the addition of the heparinized blood to the cryoprotectant solution.
3. Dispense 0.5 mL aliquots of blood suspension into 1.0 to 2.0 mL sterile plastic screw-capped cryovials. Place the vials in a controlled rate freezing unit. From room temperature, cool the vials at -1C°/min to -40°C. If the freezing unit can compensate for the heat fusion, maintain rate at -1°C/min through this phase. At -40°C, plunge the vials into liquid nitrogen. Alternatively, place the vial in a Mr. Frosty freezing container. Place the container at -80°C for minimum 4

hours and then plunge vials into liquid nitrogen.

4. To thaw a frozen ampule, place in a 35°C ± 2°C water bath, until thawed (2-3 min). Immerse the vial just sufficient to cover the frozen material. Do not agitate the ampule.
5. Immediately after thawing, aseptically remove the contents of the ampule with a syringe and inoculate an uninfected animal. Follow the protocol for in vivo propagation and maintenance in the Product Information Sheet. 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 PRA-398)

References

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

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