Babesia microti, Strain Naushon

Catalog No. NR-44072

For research use only. Not for human use.

Contributor:

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Manufacturer:

BEI Resources

Product Description:

Protozoa Classification: *Apicomplexa*, *Babesia*
Species: *Babesia microti*
Strain: Naushon
Original Source: *Babesia microti* (*B. microti*), strain Naushon was originally isolated in 1986 from a tick (*Ixodes scapularis*) collected on Naushon Island, Massachusetts, USA.[1]
Comment: *B. microti*, strain Naushon was deposited to BEI Resources as a reference strain for an ongoing genome sequencing project.[1]

Babesia species are intraerythrocytic protozoan parasites of the phylum *Apicomplexa* that are the causal agents of babesiosis, which is transmitted to both humans and mammals by infected ixodid ticks.[2] Infection with *Babesia* species is usually asymptomatic or can result in mild flu-like symptoms that subside within a few days. Severe cases featuring acute anemia, thrombocytopenia, organ failure, or even death may occur among the elderly, splenectomized and immunocompromised individuals.[2]

In the United States, *B. microti* is the most commonly identified etiologic agent of human babesiosis and is regionally endemic to the Northeastern and Upper Midwestern states, with transmission occurring via the *Ioxides scapularis* vector.[2]

Material Provided:

Each vial of NR-44072 contains approximately 0.5 mL of *B. microti* infected hamster blood in Alsever's solution containing 10% glycerol. Please see Appendix I for cryopreservation instructions and component details.

Packaging/Storage:

NR-44072 was packaged aseptically in screw-capped plastic cryovials and is provided frozen on dry ice. The product should be stored at cryogenic temperature (-130°C or colder), preferably in the vapor phase of a liquid nitrogen freezer. If liquid nitrogen storage facilities are not available, frozen cryovials may be stored at -70°C or colder for approximately one week. Note: Do not under any circumstances store vials at temperatures warmer than -70°C. Storage under these conditions will result in the death of the culture.

To insure the highest level of viability, the culture should be initiated immediately upon receipt. Any warming of the product during shipping and transfer must be avoided, as this will adversely affect the viability of the product. For transfer between freezers and for shipping, the product may be placed on dry ice for brief periods, although use of a portable liquid nitrogen carrier is preferred. Please read the following recommendations prior to using this material.

Growth Conditions:

In vivo, Golden Syrian hamster
Inoculation:

1. To increase the rate of infection the hamster should be primed by treatment with cortisone (2 mg/day/hamster) or cyclophosphamide (100 to 150 mg/kg) 1 to 3 days prior to inoculation with *B. microti*.
2. On the day of inoculation, thaw a frozen ampule of NR-44072 in a 35°C water bath for approximately 2 to 3 minutes.
3. Remove the contents of the ampule using a 1.0 mL syringe equipped with a 27 gauge 1/2 inch needle.
4. Inject the entire contents of the vial intraperitoneally into the hamster.
5. Monitor the infection at 2 to 3 day intervals by microscopic examination of blood films stained with 5% Giemsa solution.

Assessment of infection:

1. Count the number of infected red blood cells (RBC) versus the total number of red blood cells under oil immersion and determine the % parasitemia:

   % parasitemia = (Infected RBC/Total RBC) X 100

   Note: A minimum of 500 red blood cells should be counted.
2. When the level of parasitemia is between 2% and 5%, the organism should be passaged. This will normally occur 1 to 3 weeks post-inoculation, but the rate of infection may vary.
   Note: The level of parasitemia before the host succumbs is dependent on the strain that is used. Monitoring on a periodic basis will alert the experimenter when the strain should be passaged.

Passaging:

1. Immunosuppress a determined number of hamsters with cortisone or cyclophosphamide, as described in the inoculation section above, 1 to 3 days prior to inoculation with *B. microti*.
2. One day following inoculation, anesthetize the first infected hamster by CO_2/O_2 inhalation. Collect the blood by orbital bleeding using an anticoagulant such as Yaeger's anticoagulant solution (Appendix I) or EDTA.
3. Inject 0.5 mL of the infected blood suspension into each new hamster.
4. Monitor parasitemia as described above and passage as needed.

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Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: *Babesia microti*, Strain Naushon, NR-44072."

Biosafety Level: 2

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories. 5th ed. Washington, DC: U.S. Government Printing Office, 2009; see www.cdc.gov/biosafety/publications/bmbl5/index.htm.

Disclaimers:

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References:

1. Ben Mamoun, C., Personal Communication.
2. Leiby, D. A. "Transfusion-Transmitted *Babesia* spp.: Bull's-Eye on *Babesia microti*." Clin. Microbiol. Rev. 24 (2011): 14-28. PubMed: 21233506.
3. Piesman, J., et al. "Concurrent *Borrelia burgdorferi* and *Babesia microti* Infection in Nymphal *Ixodes dammini*." J. Clin. Microbiol. 24 (1986): 446-447. PubMed: 3760136.
4. Uilenberg, G. "*Babesia* – A Historical Overview." Vet. Parasitol. 138 (2006): 3-10. PubMed: 16513280.
5. Vannier, E. and P. J. Krause. "Human Babesiosis." N. Engl. J. Med. 366 (2012): 2397-2407. PubMed: 22716978.

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APPENDIX I: CRYOPRESERVATION

1. Prepare a 30% (v/v) sterile glycerol solution in Alsever's solution (see below).
2. Dispense 0.5 mL of anticoagulant solution into a 15 mL test tube. Add to the anticoagulant blood collected by orbital bleeding from hamsters 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 30% glycerol solution. Note that blood should be mixed with 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 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 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 -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 to 37°C water bath, until thawed (2 to 3 min). Immerse the ampule just sufficient to cover the frozen material. Do not agitate the ampule.
6. Immediately after thawing, remove the contents of the ampule aseptically with a syringe and inoculate an immunosuppressed hamster. Follow the protocol for *in vivo* propagation in the Growth Conditions section of the Product Information Sheet.

Alsever's Solution*

Sodium chloride	4.2 g
Trisodium citrate dihydrate ($Na_3citrate•2H_2O$)	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.
*This solution can be obtained from Sigma-Aldrich® (catalog number A3551).

Yaeger's anticoagulant solution

Sodium citrate	1.33 g
Citric acid	0.47 g
Dextrose	3.00 g
Sodium heparin	0.20 g
Glass distilled H_2O to	100.0 mL

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