Product Information Sheet for NR-12311

Babesia duncani, Strain WA1

Catalog No. NR-12311

For research use only. Not for use in humans.

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Manufacturer:
BEI Resources

Product Description:
Protozoa Classification: *Babesiidae*, *Babesia*
Species: *Babesia duncani* (also referred to as WA1-type *Babesia*[1])
Strain: WA1
Original Source: *Babesia duncani (B. duncani)*, strain WA1 was isolated in 1991 from human blood from the first reported case of babesiosis acquired in Washington State.[1]
Comment: *B. duncani,* strain WA1 was shown to be morphologically similar to, but molecularly and physiologically distinct from, *B. microti* and was subsequently described as the novel species *B. duncani* following more detailed ultrastructural and genotypic characterization.[1,2,3]

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.[4,5] 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.[4,5] The majority of human cases of babesiosis in the United States are caused by *B. microti*, while *B. divergens* is the primary cause of babesiosis in Europe, though human infections caused by *B. divergens*-like parasites in the United States have been reported.[5,6,7,8] *B. duncani* infections in the United States have occurred through both tickborne and blood transfusion routes.[9]

In mouse and hamster models of infection, *B. duncani* causes acute disease with a sudden and severe increase in parasitemia resulting in death.[10]

Material Provided:
Each vial of NR-12311 contains approximately 0.5 mL of *B. duncani*-infected hamster blood in Alsever's solution containing 10% glycerol. Please refer to Appendix I for cryopreservation instructions and component details.

Packaging/Storage:
NR-12311 was packaged aseptically in screw-capped plastic cryovials and is provided frozen on dry ice. The product should be stored at -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 ensure 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. On the day of inoculation, thaw a frozen ampule of NR-12311 in a 35°C water bath for approximately 2 to 3 minutes.
2. Remove the contents of the ampule using a 1.0 mL syringe equipped with a 27 gauge 1/2 inch needle.
3. Inject the entire contents of the vial intraperitoneally into an uninfected hamster.
4. Monitor infection on a daily basis or at 2-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 / RBC) × 100.

   Note: A minimum of 500 red blood cells should be counted.
2. When the level of parasitemia is ≥ 10%, the strain 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. 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.
2. Inject 0.5 mL of the infected blood suspension into each new hamster.
3. Monitor parasitemia as described above and passage as needed.

Please see Appendix I for cryopreservation instructions.

Citation:
Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: *Babesia duncani*, Strain WA1, NR-12311."

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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. 6th ed. Washington, DC: U.S. Government Printing Office, 2020; see www.cdc.gov/biosafety/publications/bmbl5/index.htm.

Disclaimers:

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References:

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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
Distilled H_2O to	100.0 mL

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