Babesia duncani, Strain WA1

Catalog No. NR-12311

For research use only. Not for human use.

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Product Description:
Protozoa Classification: *Babesiidae*, *Babesia*
Species: *Babesia duncani*
Strain: WA1
Original Source: *Babesia duncani* (*B. duncani*), strain WA1 was isolated 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*.[2]

Babesia sp. are tick-borne intraerythrocytic protozoan parasites of the phylum *Apicomplexa* that are the causal agents of babesiosis. *B. microti* is the most commonly identified etiologic agent of human babesiosis and is endemic in the northeastern and midwestern states. In 1991, an isolate from a patient in Washington was shown to be morphologically similar to *B. microti* but different both at the molecular and biological level.[1,2] This isolate, formerly known as WA1-type *Babesia*, was subsequently described as the novel species *B. duncani* following more detailed ultrastructural and genotypic characterization.[3]

Material Provided:
Each vial of NR-12311 contains approximately 0.5 mL of blood from infected hamster in cryopreservative. Please see Appendix I below for cryopreservation instructions.

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 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. Thaw a frozen ampule 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) X 100.

2. 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.)
3. 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 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.)

Passaging:
1. In a laminar flow hood ventilated to the outside, add one capful of 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 insert 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.
2. Add 0.5 mL of anticoagulant solution (see Appendix II) to a 5.0 mL syringe equipped with a 27 gauge 1/2 inch needle. Puncture the heart of the hamster and move the plunger of the syringe back and forth several times to distribute the anticoagulant.
3. 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.
4. 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 cotton ball

moisten with alcohol (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.

5. Inject 0.5 mL of the infected blood suspension into each uninfected hamster.
6. Monitor parasitemia and passage as needed.

Note: Hamsters may be primed for faster infection by treatment with Cortisone (2 mg/day/hamster) 1 to 3 days prior to inoculation.

Citation:

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

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.

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References:

1. Quick, R. E., et al. "Babesiosis in Washington State: A New Species of *Babesia*?" Ann. Intern. Med. 119 (1993): 284-290. PubMed: 8328736.
2. Thomford, J. W., et al. "Cultivation and Phylogenetic Characterization of a Newly Recognized Human Pathogenic Protozoan." J. Infect. Dis. 169 (1994): 1050-1056. PubMed: 8169390.
3. Conrad, P. A., et al. "Description of *Babesia duncani* n. sp. (Apicomplexa: Babesiidae) from Humans and Its Differentiation from other Piroplasms." Int. J. Parasitol. 36 (2006): 779-789. PubMed: 16725142.

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APPENDIX I: CRYOPRESERVATION

1. Prepare a 30% (v/v) sterile glycerol solution in Alsever's solution (see below).
2. Draw approximately 0.5 mL of anticoagulant solution (Yaeger's (see Appendix II) 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. Do not use if any clotting has occurred. After mixing, the final concentration of cryoprotectant solution will be 10% (v/v). Place the mixture 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 0.5 mL aliquots into 1.0 to 2.0 mL sterile plastic screw-capped cryovials. 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 to 3 min.). Immerse the ampule just sufficient to cover the frozen material. Do not agitate the ampule.
7. Immediately after thawing, remove the contents of the ampule aseptically with a syringe and inoculate an uninfected hamster. Follow the protocol for in vivo culture. The course of infection may be longer or shorter than usual depending on percent recovery of the parasite from the frozen state.

Alsever's Solution*

Sodium chloride	4.2 g
Trisodium citrate dihydrate (Na_3 citrate•$2H_2O$)	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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