

***Babesia duncani*, WA1 (in vitro)**

Catalog No. NR-50440

For research use only. Not for human use.

Contributor:

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Manufacturer:

BEI Resources

Product Description:

Protozoa Classification: Apicomplexa, *Babesia*

Species: *Babesia duncani* (also referred to as WA1-type *Babesia*¹)

Strain: WA1

Original Source: *Babesia duncani* (*B. duncani*), WA1 was isolated from human blood from the first reported case of babesiosis acquired in Washington, USA, and adapted to continuous *in vitro* culture in human erythrocytes.^{2,3}

Comment: *B. duncani*, WA1 was shown to be morphologically similar to, but molecularly and physiologically distinct from, *B. microti*.¹⁻³

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.⁵⁻⁸ *B. duncani* infections in the United States have occurred through both tickborne and blood transfusion routes.⁹

Material Provided:

Each vial of NR-50440 contains approximately 0.5 mL of infected human erythrocytes in Glycerolyte 57 solution. Please see Appendix I for cryopreservation instructions.

Packaging/Storage:

NR-50440 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:

Babesia Growth Medium (Appendix II)

Human erythrocytes (Appendix III)

Incubation:

Temperature: 37°C

Atmosphere: Humidified atmosphere of 93% N₂, 5% CO₂, 2% O₂

Propagation:

1. Place the frozen vial in a 35°C to 37°C water bath until the culture is completely thawed. Transfer the vial to a biological safety hood and wipe the outside surface of the vial with 70% ethanol.
2. Immediately after thawing, aseptically transfer the contents of the vial to a sterile 50 mL conical centrifuge tube using a 1 mL pipette.
3. Add dropwise a 12% sodium chloride (NaCl) solution to reach approximately a 1:5 ratio of NaCl to cell mixture (approximately 0.2x the original culture volume). Allow the vial to incubate for 5 minutes at room temperature.
4. Using a 10 mL pipette, add dropwise while shaking 10 volumes of a 1.5% NaCl solution (10:1 ratio of NaCl to original culture volume).
5. Centrifuge at 400 x g for 5 minutes. Remove the supernatant, leaving approximately 0.5 mL to 1 mL of supernatant in the tube. Resuspend the cells by gently swirling the tube.
6. Add dropwise while shaking 10 volumes of growth medium. Centrifuge at 400 x g for 5 minutes and carefully remove the supernatant.
7. Add 5 mL of growth medium (warmed to 37°C) and transfer the culture to a vented-cap 25-cm² cell culture flask (T-25).
8. For continuous culture, add uninfected donor red blood cells to a 10% hematocrit solution.
9. Gently aerate the culture with a 95% air, 5% CO₂ mixture through a sterile 2 mL pipette.
10. Incubate the flask at 37°C. Monitor the infection daily by microscopic examination of blood films stained with a 5% Giemsa solution. Subculture when the culture reaches ≥ 6% parasitemia.

Assessment of infection:

1. To determine parasitemia, prepare thin smears of 1 to 3 µL of cell culture samples on microscopic slides. Fix in methanol, allow to air dry. Stain with a 5% Giemsa solution, allowing the slides to incubate in the stain for 40 minutes. Prepare fresh Giemsa stain on a daily basis.
2. Examine the slides under a microscope at 1000x magnification for the presence of intracellular parasite forms.
3. 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) × 100

Note: A minimum of 500 red blood cells should be counted.

Maintenance:

1. When the culture is at or near peak density, carefully aspirate the supernatant with a sterile, unplugged Pasteur pipet under vacuum, removing as much of the supernatant as possible without aspirating the red blood cells and determine parasitemia.
2. Carefully remove a small cell sample for microscopic examination by Giemsa staining.
3. To the flask, gently add 5 to 10 mL of sterile growth medium warmed to 37°C in a water bath. Mix the media and red blood cells by gently swirling. Aerate the flask for 30 seconds with a 2% O₂, 5% CO₂ and 93% N₂ gas mixture through a sterile 2 mL pipette, tighten the cap and incubate at 37°C.
4. Monitor the culture every 2 to 3 days. Subculture when the culture reaches ≥ 6% parasitemia. Alternatively, add uninfected RBC every 3 days to increase the parasitemia within the same culture flask.

Note: During continuous incubation, the growth medium of *B. duncani*, WA1 may change color and range from bright red to almost black as a result of oxygen utilization by the parasites and deoxygenation of hemoglobin.¹⁰

Please refer to Appendix I for cryopreservation and Appendix III for preparation of human erythrocytes instructions.

Citation:

Acknowledgment for publications should read “The following reagent was obtained through BEI Resources, NIAID, NIH: *Babesia duncani*, WA1 (*in vitro*), NR-50440.”

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:

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APPENDIX I: CRYOPRESERVATION

1. Harvest *Babesia* cultures from multiple flasks using a pipette and transfer the cell suspensions to 15 mL or 50 mL plastic centrifuge tubes. Cultures should be well established and growing vigorously with a parasitemia $\geq 7\%$.
2. Centrifuge at 1300 x g for 5 minutes at room temperature.
3. Wash the pellet once with 10 or more volumes of *Babesia* growth medium. Centrifuge the cell suspension at 1800 RPM for 5 minutes. Remove the supernatant, leaving enough supernatant to resuspend the pellet. Estimate the volume of the remaining cell suspension.
4. To the volume of packed red blood cells, slowly add dropwise one volume of cold (4°C) Glycerolyte 57 solution (Baxter Healthcare Corporation 4A7831, or equivalent). Allow to incubate for 5 minutes at room temperature.
5. Add dropwise an additional 4 volumes of cold Glycerolyte 57 solution to the pellet and mix well.
6. Dispense 0.5 mL aliquots into 1 to 2 mL sterile plastic screw-capped vials for cryopreservation.
7. 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.
8. Store in either the vapor or liquid phase of a nitrogen refrigerator (-130°C or colder).

APPENDIX II: *BABESIA* GROWTH MEDIUM

1. Prepare a 10 mM hypoxanthine and 1.5 mM thymidine stock solution in distilled, deionized water and filter sterilize using a 0.22 µm filter. An equivalent prepared solution may also be used. The final concentration in the *Babesia* Growth Medium is 200 µM hypoxanthine and 32 µM thymidine.
2. Aseptically prepare the *Babesia* Growth Medium (see recipe below), filter sterilize using a 0.22 µm filter and store at 4°C. Use prepared medium within two weeks. Adjust the complete medium pH to 7.2, if needed.

Babesia Growth Medium

HL-1™ Chemically Defined, Serum-Free Medium (Lonza 77201)	75 mL
Human Serum Type A Positive	20 mL
HB 101® supplement (Irvine Scientific® T151)	1 mL
200 mM L-glutamine stock solution (ATCC® 30-2214™)	1 mL
10 mM Hypoxanthine/1.5 mM Thymidine stock solution	2 mL

Note: To prevent culture contamination, Penicillin-Streptomycin-Amphotericin B (Antibiotic/Antimycotic) Solution (ATCC® PCS-999-002™) may be added to a final concentration of 100 IU/mL penicillin, 100 µg/mL streptomycin and 0.25 µg/mL amphotericin B. Gentamicin may also be added to a final concentration of 100 µg/mL.

APPENDIX III: PREPARATION OF HUMAN ERYTHROCYTES

1. Prepare the Puck's Saline Glucose (PSG) medium (see recipe below), mix well, adjust pH to 7.2, and adjust the volume to 1 L with distilled, deionized water. Filter sterilize using a 0.22 µm filter and store at 4°C.
2. Prepare the PSG+G solution (see recipe below), mix well, filter sterilize using a 0.22 µm filter and store at 4°C.

Puck's Saline Glucose Medium

CaCl ₂ • 7H ₂ O	0.016 g
KCl	0.40 g
KH ₂ PO ₄	0.15 g
MgSO ₄ • 7H ₂ O	0.15 g
NaCl	8.0 g
Na ₂ HPO ₄ • 7H ₂ O	0.29 g
D-glucose	1.10 g
Phenol red	0.005 g
Distilled, deionized water to	1 L

PSG+G Solution

Puck's Saline Glucose Medium	500 mL
D-glucose	10 g
Antibiotic/Antimycotic Solution (ATCC® PCS-999-002™)	5 mL

3. Aseptically, wash donor blood three times by centrifugation at 600 to 800 x g for 15 minutes at 4°C in sterile phosphate buffered saline (PBS) without calcium or magnesium (ATCC® 30-2200™), adjusted to contain 15 mM ethylenediaminetetraacetic acid (EDTA).
4. After each wash, aseptically remove the supernatant, consisting of the plasma and buffy (lymphocyte) layers, from the top of the red blood cell (erythrocyte) pellet.
5. After the last wash, aseptically resuspend human erythrocytes in sterile PSG+G solution at a concentration of 50% erythrocytes. The human erythrocytes in PSG+G solution may be stored at 4°C until use, for a maximum of two weeks.