Babesia duncani, Strain WA1, Clone BdWA1-301 *(in vitro)*

Catalog No. NR-59103

For research use only. Not for use in humans.

Contributor:

Choukri Ben Mamoun, Ph.D., Professor of Medicine (Infectious Diseases) and of Microbial Pathogenesis, Yale University, New Haven, Connecticut, USA

Manufacturer:

BEI Resources

Product Description:

Protozoa Classification: *Apicomplexa, Babesia*
Species: *Babesia duncani*
Strain: WA1, Clone BdWA1-301 *(in vitro)*
Original Source: *Babesia duncani (B. duncani)*, strain WA1, clone BdWA1-301 was derived through three consecutive limiting dilution cloning events of strain WA1 performed *in vitro*.[1] Strain WA1 was isolated in 1991 from human blood from the first reported case of babesiosis acquired in Washington State.[2]
Comments: The complete genome of *B. duncani*, strain WA1 has been sequenced (GenBank: JALLKP000000000).

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.[3,4] 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.[3,4] 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.[4,5,6,7] *B. duncani* infections in the United States have occurred through both tickborne and blood transfusion routes.[8]

B. duncani, strain WA1, clone BdWA1-301 has demonstrated *in vitro* and *in vivo* parasitemia comparable to that of the parental WA1 strain (BEI Resources NR-12311).[1]

Material Provided:

Each vial of NR-59103 contains approximately 0.5 mL of *B. duncani*-infected human blood in Glycerolyte 57 (1:5). Please refer to Appendix I for cryopreservation instructions.

Packaging/Storage:

NR-59103 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:

DMEM/F12-Based *Babesia* Growth Medium[1] (Appendix II)
Human whole blood, Type O+ (Appendix III)
Incubation:
Temperature: 37°C
Atmosphere: Humidified atmosphere of 93% N_2, 5% CO_2, 2% O_2
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 0.2× 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 1.6% NaCl solution (10:1 ratio of NaCl to original culture volume).
5. Centrifuge at 625 × 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 625 × 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 non-vented cap 25-cm^2 cell culture flask (T-25).
8. For continuous culture, add uninfected donor red blood cells (RBCs) to a 5% hematocrit (HT) suspension every 2 to 3 days.
9. Gently aerate the culture with a 93% N_2, 5% CO_2, 2% O_2 gas mixture through a sterile, cotton-plugged Pasteur pipet and then quickly tighten the cap. Incubate the flask at 37°C.
10. Monitor the parasitemia daily by microscopic examination of blood films stained with a Giemsa solution.

Assessment of parasitemia:

1. To determine parasitemia of the culture, prepare thin smears of 3 µL to 5 µL of cell culture samples on microscopic slides, fix in methanol and allow to air dry. Stain with a 5% Giemsa solution, allowing the slides to incubate in the stain for 40 minutes. Prepare fresh Giemsa solution on a daily basis.

2. Examine the slides under a microscope at 1000× magnification for the presence of intracellular parasite forms.
3. Count the number of infected RBCs versus the total number of RBCs under oil immersion and determine the % parasitemia:
   % parasitemia = (Infected RBC/Total RBC) × 100
   Note: For a more accurate determination of parasitemia, a minimum of 500 RBCs should be counted.

Maintenance:

1. Carefully remove the flask with infected culture from the 37°C incubator without disturbing the RBCs and place it inside a biosafety cabinet.
2. Carefully remove the supernatant with a sterile, unplugged Pasteur pipet under vacuum, or using a sterile pipette. Remove as much of the supernatant as possible without removing the cells.
3. Remove a small cell sample for microscopic examination by Giemsa staining.
4. To the culture flask, gently add prewarmed (37°C) sterile growth medium and uninfected donor RBCs, as needed, for a total of 5% hematocrit. Mix the medium and the cells inside the flask by gentle swirling.
5. Aerate the culture flask with a 93% N_2, 5% CO_2, 2% O_2 gas mixture through a sterile pipette, tighten the cap and incubate the flask in a 37°C incubator.

Note: For rapid increase of parasitemia, changing of the culture medium daily is required for *Babesia*-infected erythrocyte cultures. Subculture should be performed when the culture is stable and parasitemia reaches 6%.

Please refer to Appendix I for cryopreservation, Appendix II for complete medium preparation instructions and Appendix III for preparation of human donor erythrocytes instructions.

Citation:

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: *Babesia duncani*, Strain WA1, Clone BdWA1-301 *(in vitro)*, NR-59103."

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 (BMBL). 6th ed. Washington, DC: U.S. Government Printing Office, 2020.

All blood cultures should be handled with appropriate safety precautions necessary for the handling of bloodborne pathogens. Personnel must be trained in accordance with their institutional policy regarding bloodborne pathogens.

Disclaimers:

You are authorized to use this product for research use only. It is not intended for human use.

Use of this product is subject to the terms and conditions of the BEI Resources Material Transfer Agreement (MTA). The MTA is available on our Web site at www.beiresources.org.

While BEI Resources uses reasonable efforts to include accurate and up-to-date information on this product sheet, neither ATCC® nor the U.S. Government makes any warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. Neither ATCC® nor the U.S. Government warrants that such information has been confirmed to be accurate.

This product is sent with the condition that you are responsible for its safe storage, handling, use and disposal. ATCC® and the U.S. Government are not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to ensure authenticity and reliability of materials on deposit, the U.S. Government, ATCC®, their suppliers and contributors to BEI Resources are not liable for damages arising from the misidentification or misrepresentation of products.

Use Restrictions:

This material is distributed for internal research, non-commercial purposes only. This material, its product or its derivatives may not be distributed to third parties. Except as performed under a U.S. Government contract, individuals contemplating commercial use of the material, its products or its derivatives must contact the contributor to determine if a license is required. U.S. Government contractors may need a license before first commercial sale.

References:

1. Singh, P., A. C. Pal and C. Ben Mamoun. "An Alternative Culture Medium for Continuous *In Vitro* Propagation of the Human Pathogen *Babesia duncani* in Human Erythrocytes." Pathogens 22 (2022): 599. PubMed: 35631120.
2. 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.
3. Leiby, D. A. "Transfusion-Transmitted *Babesia* spp.: Bull's-Eye on *Babesia microti*." Clin. Microbiol. Rev. 24 (2011): 14-28. PubMed: 21233506.
4. Vannier, E. and P. J. Krause. "Human Babesiosis." N. Engl. J. Med. 366 (2012): 2397-2407. PubMed: 22716978.
5. Beattie, J. F., M. L. Michelson and P. J. Holman. "Acute Babesiosis Caused by *Babesia divergens* in a Resident of Kentucky." N. Engl. J. Med. 347 (2002): 697-698. PubMed: 12200568.
6. Herwaldt, B. L., et al. "A Fatal Case of Babesiosis in Missouri: Identification of Another Piroplasm that Infects Humans." Ann. Intern. Med. 124 (1996): 643-650. PubMed: 8607592.
7. Herwaldt, B. L., et al. "*Babesia divergens*-Like Infection, Washington State." Emerg. Infect. Dis. 10 (2004): 622-629. PubMed: 15200851.
8. Herwaldt, B. L., et al. "Transfusion-Associated Babesiosis in the United States: A Description of Cases." Ann. Intern. Med. 155 (2011): 509-519. PubMed: 21893613.

BEI Resources
www.beiresources.org

E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898

Support Provided by NIAID

APPENDIX I: CRYOPRESERVATION

1. Harvest *Babesia* cultures from multiple flasks using sterile pipettes 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 ≥ 6%.
2. Centrifuge at 625 × g for 5 minutes at room temperature.
3. Wash the pellet once with 10 or more volumes of incomplete DMEM/F12 medium. Centrifuge the cell suspension at 625 × g for 5 minutes. Remove most of the supernatant, leaving enough supernatant to resuspend the pellet. Estimate the volume of the pellet.
4. To the volume of packed red blood cells, slowly add dropwise one volume of cold (4°C) Glycerolyte 57 solution (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 to 2 days and then plunge vials into liquid nitrogen.
8. Store the frozen vials in either the vapor or liquid phase of a nitrogen refrigerator (-130°C or colder).

APPENDIX II: DMEM/F12-BASED *BABESIA* GROWTH MEDIUM

1. Aseptically prepare the DMEM/F12-based *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.

DMEM/F12-Based Babesia Growth Medium
DMEM/F12 Medium (Lonza™ BE04-687F or equivalent) adjusted to contain:
20% Heat-inactivated fetal bovine serum (HIFBS)
4 mM L-glutamine (ATCC® 30-2214™)
100 µM Hypoxanthine
16 µM Thymidine

Note: To prevent culture contamination, Penicillin-Streptomycin-Amphotericin B (Antibiotic/Antimycotic) Solution (ATCC® PCS-999-002™, or equivalent) 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	
$CaCl_2 \cdot 7H_2O$	0.016 g
KCl	0.4 g
KH_2PO_4	0.15 g
$MgSO_4 \cdot 7H_2O$	0.15 g
NaCl	8 g
$Na_2HPO_4 \cdot 7H_2O$	0.29 g
D-glucose	1.1 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 × g for 15 minutes at 4°C in RPMI 1640 medium.
4. After each wash, aseptically remove the supernatant, consisting of the plasma and buffy (leukocyte) layers located on the top of the RBC (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.

BEI Resources
www.beiresources.org
E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898