Babesia divergens, Strain Rouen 87 (*in vitro*)

Catalog No. NR-52008

For research use only. Not for use in humans.

Contributor:
Dr. Stéphane Delbecq, Researcher, Center of Structural Biochemistry, University of Montpellier, Montpellier, France, French National Center for Scientific Research (CNRS) and French National Institute for Health and Medical Research (INSERM), France

Manufacturer:
BEI Resources

Product Description:
Protozoa Classification: *Apicomplexa, Babesia*
Species: *Babesia divergens*
Strain: Rouen 87 (also referred to as Rouen 1987)[1]
Original Source: *Babesia divergens (B. divergens)*, strain Rouen 87 isolated in 1987 from blood of a human patient in France.[1]
Comments: The complete genome of *B. divergens*, strain Rouen 87 has been sequenced (GenBank: CCSG00000000.2).[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.[4,5,6] In humans, babesiosis 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] Four species of *Babesia* confirmed to infect humans have been identified: *B. microti, B. divergens, B. duncani and B. venatorum.*[3] *B. microti* has a worldwide distribution and is the most commonly identified etiologic agent of human babesiosis in the United States.[3] In Europe, *B. divergens* is the primary cause of babesiosis in humans and cattle.[4]

No reliable long-term *in vitro* culture system is available for *B. microti*, which requires propagation in rodents. The availability of continuous *in vitro* culture techniques for *B. duncani* and *B. divergens*, however, provides the opportunity to examine the basic biology of *Babesia*, host-pathogen interactions, immune factors triggered by infection, development of transgenic strains and *in vitro* drug screening studies.

Material Provided:
Each vial of NR-52008 contains approximately 0.5 mL of *B. divergens*-infected human blood in Glycerolyte 57 (1:5). Please refer to Appendix I for cryopreservation instructions.

Packaging/Storage:
NR-52008 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:
RPMI 1640 medium adjusted to contain 10% (v/v) heat-inactivated human serum (pooled Type A), 25 mM HEPES, 2 mM L-glutamine, 2 g/L D-glucose, 27 µg/mL hypoxanthine, 4.4 g/L sodium bicarbonate and 5 µg/mL gentamicin (optional); (Appendix II)
Human erythrocytes (Appendix III)
Incubation:
Temperature: 37°C
Atmosphere: 90% N_2, 5% CO_2, 5% 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 1000 × 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 1000 × 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 1% to 2% hematocrit solution.
9. Gently aerate the culture with a 90% N_2, 5% CO_2, 5% O_2 mixture through a sterile, cotton-plugged Pasteur pipet. Incubate the flask at 37°C.

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

10. Incubate the flask at 37°C. Monitor the infection daily by microscopic examination of blood films stained with a 5% Giemsa solution.

Assessment of infection:

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, 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 1000× 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. Remove the flask with infected culture from the 37°C incubator and place inside a biosafety cabinet.
2. Carefully remove the supernatant with a sterile, unplugged Pasteur pipet under vacuum. Remove as much of the supernatant as possible without taking the cells.
3. Add 25 mL of sterile warm (37°C) complete medium to the flask, gently mix and aerate, then quickly tighten the cap and place the flask in the 37°C incubator until the next change of medium.

Note: Changing of the culture medium daily is required for *Babesia*-infected erythrocyte cultures.

Please refer to Appendix I for cryopreservation, Appendix II for complete medium preparation instructions 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 divergens*, Strain Rouen 87 *(in vitro)*, NR-52008."

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. Precigout, E., et al. "Analysis of Immune Responses of Different Hosts to *Babesia divergens* Isolates from Different Geographic Areas and Capacity of Culture-Derived Exoantigens to Induce Efficient Cross-Protection." Infect. Immun. 59 (1991): 2799-2805. PubMed: 1713201. Erratum in: Infect. Immun. 60 (1992): 1728.
2. Cuesta, I., et al. "High-Quality Draft Genome Sequence of *Babesia divergens*, the Etiological Agent of Cattle and Human Babesiosis." Genome Announc. 2 (2014): e01194-14. PubMed: 25395649.
3. González, L. M., et al. "Comparative and Functional Genomics of the Protozoan Parasite *Babesia divergens* Highlighting the Invasion and Egress Processes." PLoS Negl. Trop. Dis. 13 (2019): e0007680. PubMed: 31425518.
4. Kumar, A., J. O'Bryan and P. J. Krause. "The Global Emergence of Human Babesiosis." Pathogens 10 (2021): 1447. PubMed: 34832603. Erratum in: Pathogens 11 (2022): 607.
5. Renard, I. and C. B. Mamoun. "Treatment of Human Babesiosis: Then and Now." Pathogens 10 (2021): 1120. PubMed: 34578153.
6. Yang, Y., et al. "Emerging Human Babesiosis with "Ground Zero" in North America." Microorganisms 9 (2021): 440. PubMed: 33672522.

Support Provided by NIAID

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 ≥ 4%.
2. Centrifuge at 1000 × g for 5 minutes at room temperature.
3. Wash the pellet once with 10 or more volumes of incomplete RPMI 1640 medium. Centrifuge the cell suspension at 1000 × g 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 (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 in either the vapor or liquid phase of a nitrogen refrigerator (-130°C or colder).

APPENDIX II: *BABESIA* GROWTH MEDIUM

1. Incomplete Medium: Used for many applications involving wash steps during preparation of parasites for culture or assay. The incomplete medium consists of RPMI 1640 medium supplemented with the following components[1]:

Incomplete Medium	
RPMI 1640 medium[2,3]	
Sodium bicarbonate ($NaHCO_3$)[4]	2.4 g/L
L-Glutamine	2 mM
HEPES [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid]	25 mM
D-Glucose[5]	2 g/L
Hypoxanthine	27 µg/mL
Gentamicin (optional)	5 µg/mL

[1]Prepare sterile stock solutions at concentrations that are easily diluted into the liquid medium to obtain the appropriate user concentrations and add aseptically. Ready-made stock solutions for many of the components are available from numerous manufacturers.
[2]RPMI 1640 medium is available from numerous manufacturers as both a powder and a sterile, prepared liquid, with or without L-glutamine and HEPES. If using powdered RPMI 1640 medium, prepare the medium following manufacturer instructions, sterile filter using a 0.22 µm filter, then aseptically add the necessary components in the appropriate concentrations.
[3]If stock solutions were not sterile or aseptic techniques were not followed, sterile-filter the medium using a 0.22 µm filter after the addition of all components. Store at 4°C.
[4]Prepared, liquid medium typically contains sodium bicarbonate while powdered medium does not. A typical concentration of sodium bicarbonate in RPMI 1640 medium is 2 g/L, though some formulations contain different amounts.
[5]A typical concentration of D-glucose in RPMI 1640 medium is 2 g/L. The option to supplement with an additional 2 g/L yields a final concentration of 4 g/L D-glucose.

2. Complete Medium: consists of incomplete medium (above) supplemented with 10% heat-inactivated human serum. If necessary, filter the complete medium with a 0.22 µm filter. Since serum tends to clog sterilizing filters, a serum pre-filter may be used first, followed by a 0.22 µm sterilizing filter.

   Note: Human serum type A is used with washed type O blood. Serum substitutes may be used; however, they may not be acceptable for all parasite strains.

BEI Resources
www.beiresources.org

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

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	0.40 g
KH_2PO_4	0.15 g
$MgSO_4 \cdot 7H_2O$	0.15 g
NaCl	8.0 g
$Na_2HPO_4 \cdot 7H_2O$	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 × 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 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.