# *Babesia duncani*

## PRA-433™

### Description

*Babesia duncani* strain WA1, Clone BdWA1-303 was derived through three consecutive limiting dilution cloning events of *Babesia duncani* strain WA1 (ATCC PRA-302) performed in vitro. This strain has demonstrated in vitro and in vivo parasitemia comparable to that of the parental WA1 strain.
**Strain designation:** WA1, Clone BdWA1-303

### Storage Conditions

**Product format:** Frozen
**Storage conditions:** -80°C or colder for 1 week, vapor phase of liquid nitrogen for long-term storage

### Intended Use

This product is intended for laboratory research use only. It is not intended for any animal or human therapeutic use, any human or animal consumption, or any diagnostic use.

### BSL 2

ATCC determines the biosafety level of a material based on our risk assessment as guided by the current edition of *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, U.S. Department of Health and Human Services. It is your responsibility to understand the hazards associated with the material per your organization's policies and procedures as well as any other applicable regulations as enforced by your local or national agencies.

ATCC highly recommends that appropriate personal protective equipment is always used when handling vials. For cultures that require storage in liquid nitrogen, it is important to note that some vials may leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vial exploding or blowing off its cap with dangerous force creating flying debris. Unless necessary, ATCC recommends that these cultures be stored in the vapor phase of liquid nitrogen rather than submersed in liquid nitrogen.

## Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at www.atcc.org.

## Growth Conditions

**Instructions for complete medium:** DMEM/F12-based *Babesia* Growth Medium and human whole blood, type O+

**Preparation of DMEM/F12-based *Babesia* Growth Medium**
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-glutimine (ATCC 30-2214)
100 µM Hypoxanthine
16 µM Thymidine

Aseptically prepare the medium, filter sterilize using at 0.22 µm filter, and store at 4°C. Use the prepared medium within two weeks. Adjust the complete medium pH to

7.2 if needed.

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.

**Preparation of Human Erythrocytes**

Puck's Saline Glucose Medium

$CaCl_2 \cdot 7H_2O$, 0.016 g
KCl, 0.4 g
$KH_2PO_4$, 0.15 g
$MgSO_4 \cdot 7\ H_2O$, 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

1. Prepare the Puck's Saline Glucose (PSG) Medium, 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, mix well, filter sterilize using a 0.22 µm filter, and store at 4°C.
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 plasmid 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.

**Temperature:** 37°C
**Atmosphere:** Microaerophilic: 2% $O_2$- 5% $CO_2$ -93% $N_2$

## Handling Procedures

### Storage and Culture Initiation

Frozen ampules packed in dry ice should either be thawed immediately or stored in liquid nitrogen. If liquid nitrogen storage facilities are not available, frozen ampoules may be stored at or below -70°C for approximately one week. **Do not under any circumstance store frozen ampules at refrigerator freezer temperatures (generally -20°C).** Storage of frozen material at this temperature will result in the death of the culture.

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 at 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 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-$mm^2$ 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 cultures with a 93% $N_2$, 5% $CO_2$, and 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 RBSs under oil immersion and determine the % parasitemia.
   % parasitema = (infected RBC/Total RBC) × 100
   Note: For a more accurate determination of parasitemia, a minimum of 500 RBCs should be counted.

**Culture 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%.

**Cryopreservation:**

1. Harvest *Babesia* cultures from multiple flask 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).

## Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Babesia duncani* (ATCC PRA-433)

## References

References and other information relating to this material are available at www.atcc.org.

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## Revision

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