Certificate of Analysis for NR-59103

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

Catalog No. NR-59103

Product Description:

Babesia duncani (B. duncani), strain WA1, clone BdWA1-301 was derived through three consecutive limiting dilution cloning events of strain WA1 performed *in vitro*. Strain WA1 was isolated in 1991 from human blood from the first reported case of babesiosis acquired in Washington State. NR-59103 was produced by cultivation of the deposited material in human Type O+ erythrocytes with DMEM/F12-based *Babesia* growth medium adjusted to contain 20% (v/v) heat-inactivated fetal bovine serum (HIFBS), 4 mM L-glutamine, 100 µM hypoxanthine, 16 µM thymidine, 100 IU/mL penicillin, 100 µg/mL streptomycin, 0.25 µg/mL amphotericin B and 25 µg/mL gentamicin. After one passage, the culture was propagated in human Type O+ erythrocytes at 37°C in sealed flasks outgassed with a blood-gas atmosphere (93% N_2, 5% CO_2, 2% O_2) and monitored for parasitemia for 10 days to produce this lot. Quality control testing was completed under propagation conditions unless otherwise noted.

Lot: 70063009 **Manufacturing Date: 08SEP2023**

TEST	SPECIFICATIONS	RESULTS
Cell Morphology[1] 7 days of infection by examination of Giemsa-stained blood smears	Report results	Infection of red blood cells visible (Figure 1)
Genotypic Analysis[2] Sequencing of internal transcribed spacer (ITS) 1, 5.8S rRNA gene, ITS 2 (~ 690 base pairs)	≥ 99% sequence identity to *B. duncani*, strain WA1 (GenBank: JALLKP00000000)	99.7% sequence identity to *B. duncani*, strain WA1 (GenBank: JALLKP010000137.1)
Level of Parasitemia (pre-freeze)[2] 10 days of infection by microscopic counts of Giemsa-stained blood smears	Report results	8.6%
Viability[1,3]	Growth	Growth
Sterility (21-day incubation)[1]		
Harpo's HTYE broth, 37°C and 26°C, aerobic[4]	No growth	No growth
Trypticase soy broth, 37°C and 26°C, aerobic	No growth	No growth
Sabouraud broth, 37°C and 26°C, aerobic	No growth	No growth
DMEM with 10% FBS, 37°C, aerobic	No growth	No growth
Sheep blood agar, 37°C, aerobic	No growth	No growth
Sheep blood agar, 37°C, anaerobic	No growth	No growth
Thioglycollate broth, 37°C, anaerobic	No growth	No growth
Mycoplasma Contamination[1] DNA Detection by PCR	None detected	None detected

[1]Testing completed on vialed, post-freeze material.
[2]Testing completed on bulk material prior to vialing and freezing.
[3]Viability of the material following cryopreservation was determined by cultivation in human Type O+ erythrocytes with DMEM/F12-based *Babesia* growth medium adjusted to contain 20% (v/v) heat-inactivated fetal bovine serum (HIFBS), 4 mM L-glutamine, 100 µM hypoxanthine, 16 µM thymidine, 100 IU/mL penicillin, 100 µg/mL streptomycin, 0.25 µg/mL amphotericin B and 25 µg/mL gentamicin at 37°C in an atmosphere of 93% N_2, 5% CO_2, 2% O_2 and examination of parasitemia every day for 1 to 2 days post-infection (12% parasitemia).
[4]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

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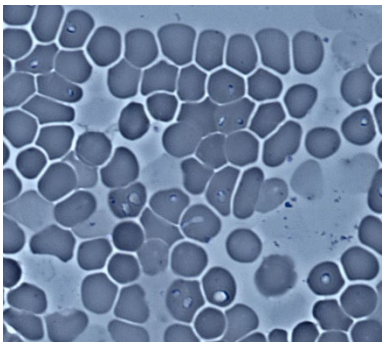
Figure 1: Cell Morphology



/Sonia Bjorum Brower/
Sonia Bjorum Brower 09 APR 2024
Technical Manager or designee, ATCC Federal Solutions

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC®'s knowledge.

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