

## Certificate of Analysis for NR-50441

Babesia sp., MO1 (in vitro)

Catalog No. NR-50441

**Product Description:** Babesia sp., MO1 was isolated in 2003 from the blood of a wild Eastern cottontail rabbit (*Sylvilagus floridanus*) on Nantucket Island, Massachusetts, USA, and adapted to continuous *in vitro* culture in human erythrocytes.

Lot<sup>1</sup>: 70002062 Manufacturing Date: 13FEB2017

TEST	SPECIFICATIONS	RESULTS
Cellular Morphology <sup>2</sup>	Report results	Pleomorphic rings and tetrads
Genotyping³ Sequencing of 18S ribosomal RNA (rRNA) gene (~ 1570 base pairs)	≥ 99% sequence identity to  **Babesia* sp., strain MO1  (GenBank: AY048113.1)	99.9% sequence identity to Babesia sp., strain MO1 (GenBank: AY048113.1) <sup>4</sup>
Functional Activity by PCR Amplification <sup>3,5</sup> 18S rRNA gene	~ 930 base pair amplicon	~ 930 base pair amplicon
Level of Parasitemia (pre-freeze) <sup>3,6</sup>	Report results	6.8%
Viability <sup>2,7</sup>	Growth	Growth
Sterility (21-day incubation) <sup>2</sup> Harpo's HTYE broth <sup>8</sup> , 37°C and 26°C, aerobic Tryptic soy broth, 37°C and 26°C, aerobic Sabouraud broth, 37°C and 26°C, aerobic DMEM with 10% FBS, 37°C, aerobic Sheep blood agar, 37°C, aerobic Sheep blood agar, 37°C, anaerobic Thioglycollate broth, 37°C, anaerobic	No growth	No growth
Mycoplasma Contamination DNA Detection by PCR	None detected	None detected

<sup>&</sup>lt;sup>1</sup>NR-50441 was produced by cultivation of the deposited material in human erythrocytes with *Babesia* Growth Medium (HL-1<sup>™</sup> Chemically Defined, Serum-Free Medium (Lonza 77201), adjusted to contain 20% Human Serum Type A Positive, 1% HB 101<sup>®</sup> supplement (Irvine Scientific<sup>®</sup> T151), 2 mM L-glutamine, 200 μM hypoxanthine, 32 μM thymidine, 100 IU/mL penicillin, 100 μg/mL streptomycin and 0.25 μg/mL amphotericin B and 100 μg/mL gentamicin) for 14 days at 35°C in an aerobic atmosphere with 5% CO₂ until the first peak of parasitemia was reached.

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**Date:** 20 SEP 2017 **Signature:** 

BEI Authentication or designee

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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<sup>&</sup>lt;sup>2</sup>Testing completed on vialed, post-freeze material.

<sup>&</sup>lt;sup>3</sup>Testing completed on bulk material prior to vialing and freezing.

<sup>&</sup>lt;sup>4</sup>Also consistent with other *Babesia* species

<sup>&</sup>lt;sup>5</sup>Primer sequences and conditions for PCR are available upon request.

<sup>&</sup>lt;sup>6</sup>Parasitemia was determined after 14 days of infection by microscopic counts of Giemsa-stained blood smears.

<sup>&</sup>lt;sup>7</sup>Viability of the material following cryopreservation was determined by inoculation in human erythrocytes in *Babesia* Growth Medium and examination of parasitemia every 1 to 3 days for 13 days post-infection (5.9% parasitemia).

<sup>&</sup>lt;sup>8</sup>Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.