Certificate of Analysis for NR-52008

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

Catalog No. NR-52008

Product Description:

Babesia divergens (B. divergens), strain Rouen 87 isolated in 1987 from blood of a human patient in France. NR-52008 was produced by cultivation of the deposited material in human erythrocytes with 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 25 µg/mL gentamicin. After three passages, the culture was propagated in human Type O erythrocytes at 37°C in sealed flasks outgassed with a blood-gas atmosphere (90% N_2, 5% CO_2, 5% O_2) and monitored for parasitemia for 17 days.

Lot: 70053901 **Manufacturing Date: 06AUG2022**

TEST	SPECIFICATIONS	RESULTS
Cell Morphology[1] 3 days of infection by examination of Giemsa-stained blood smears	Report results	Infection of red blood cells visible
Genotypic Analysis[2] Sequencing of 18S ribosomal RNA (rRNA) gene (~ 1630 base pairs)	≥ 99% sequence identity to *B. divergens*, strain Rouen 87 (GenBank: CCSG02000039.1)	99.9% sequence identity to *B. divergens*, strain Rouen 87 (GenBank: CCSG02000039.1)
Sequencing of internal transcribed spacer (ITS) 1, 5.8S rRNA gene, ITS 2 (~580 base pairs)	≥ 99% sequence identity to *B. divergens*, strain Rouen 87 (GenBank: CCSG02000039.1)	99.8% sequence identity to *B. divergens*, strain Rouen 87 (GenBank: CCSG02000039.1)[3]
Level of Parasitemia (pre-freeze)[2] 17 days of infection by microscopic counts of Giemsa-stained blood smears	Report results	4%
Viability[1,4]	Growth	Growth
Sterility (21-day incubation)[1]		
Harpo's HTYE broth, 37°C and 26°C, aerobic[5]	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]Also consistent with *Babesia capreoli*
[4]Viability of the material following cryopreservation was determined by cultivation in human Type O erythrocytes with 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 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 3 days post-infection (14% parasitemia).
[5]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

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

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/Sonia Bjorum Brower/
Sonia Bjorum Brower 24 MAY 2023
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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BEI Resources
www.beiresources.org
E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898