Certificate of Analysis for NR-51675

Borrelia miyamotoi, Strain HT31

Catalog No. NR-51675

Product Description:

Borrelia miyamotoi (B. miyamotoi), strain HT31 was isolated from the abdomen of an unfed female *Ixodes persulcatus* tick collected from vegetation between 1990 and 1992 in Shiretoko, Hokkaido, Japan. The deposited material was inoculated into Revised Barbour-Stoenner-Kelly broth and grown for two passages at 33°C in an aerobic atmosphere with 5% CO_2, and the resulting subculture was vialed and frozen. NR-51675 lot 70032899 was produced by inoculation of the deposited material into Revised Barbour-Stoenner-Kelly broth. After two passages, the culture was grown for 7 days at 33°C in an aerobic atmosphere with 5% CO_2. Broth inoculum was added to Revised Barbour-Stoenner-Kelly broth and grown for 6 days at 33°C in a microaerophilic atmosphere to produce this lot.

Lot: 70032899 **Manufacturing Date: 29JAN2020**

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology 7 days at 33°C in a microaerophilic atmosphere in Revised Barbour-Stoenner-Kelly broth	Spirochete	Spirochete
Motility (wet mount)	Report results	Motile
Genotypic Analysis		
Sequencing of 16S ribosomal RNA (rRNA) gene (1410 base pairs)	≥ 99% sequence identity to *B. miyamotoi*, strain HT31 (GenBank: AB904793.1)	100% sequence identity to *B. miyamotoi*, strain HT31 (GenBank: AB904793.1)
Purity (post-freeze)		
7 days at 33°C in an aerobic atmosphere with 5% CO_2 on Tryptic Soy agar with 5% defibrinated sheep blood	Growth consistent with colony morphology or no growth	No growth
7 days at 37°C in an aerobic atmosphere with 5% CO_2 on Tryptic Soy agar with 5% defibrinated sheep blood	No growth	No growth
Viability (post-freeze)		
Visual observation 7 days at 33°C in a microaerophilic atmosphere in Revised Barbour-Stoenner-Kelly broth	Growth	Growth
LIVE/DEAD® *Bac*Light™ Bacterial Viability	Green fluorescence visible	Green fluorescence visible (Figure 1)[1]

[1]Determined after 7 days at 33°C in an aerobic atmosphere with 5% CO_2 in Revised Barbour-Stoenner-Kelly broth with LIVE/DEAD® *Bac*Light™ Bacterial Viability Kit, 1000× magnification (Invitrogen™ L34856). Cells with a compromised membrane that are dead or dying will stain red, while cells with an intact membrane will stain green.

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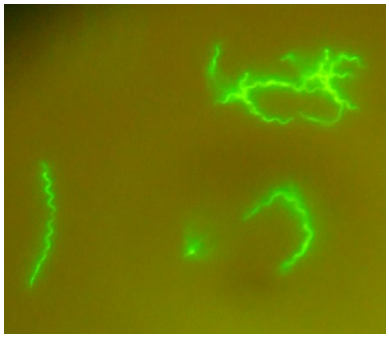
Figure 1: LIVE/DEAD® *Bac*Light™ Bacterial Viability



/Heather Couch/
Heather Couch 28 APR 2020
Program 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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