

Certificate of Analysis for NR-35358

Leptospira interrogans, Strain CSL4002 (Serovar Pomona)

Catalog No. NR-35358

This reagent is the tangible property of the U.S. Government.

Product Description:

Leptospira interrogans (L. interrogans), strain CSL4002 (serovar Pomona) was isolated from a sea lion in California, USA. NR-35358 lot 70031223 was produced by inoculation of the deposited material into EMJH semisolid agar (0.15%) and grown for 41 days at 30°C in an aerobic atmosphere. The material from this growth was passaged once in EMJH semisolid agar (0.15%) for 23 days at 30°C in an aerobic atmosphere to produce this lot.

Lot: 70031223 Manufacturing Date: 20FEB2020

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology	Spirochete	Spirochete
20 days at 33°C in an aerobic atmosphere in EMJH semisolid agar (0.15%)		
Colony morphology	Growth below the soft agar surface (Dinger's disk)	Growth below the soft agar surface (Dinger's disk) ¹ (Figure 1)
Motility (wet mount)	Report results	Motile
Genotypic Analysis		
Sequencing of 16S ribosomal RNA (rRNA) gene	≥ 99% sequence identity to	99.9% sequence identity to
(~ 1450 base pairs)	L. interrogans, strain CSL4002 (GenBank: ANMZ01000132.1)	L. interrogans, strain CSL4002 (GenBank: ANMZ01000132.1) ²
Purity		
20 days at 37°C in an aerobic atmosphere with	No growth	No growth
5% CO ₂ in Tryptic Soy agar with 5% defibrinated		
sheep blood		
Viability (post-freeze)		
Visual observation	Growth	Growth
20 days at 33°C in an aerobic atmosphere in EMJH semisolid agar (0.15%)		
LIVE/DEAD [®] <i>Bac</i> Light™ Bacterial Viability	Green fluorescence visible	Green fluorescence visible ³ (Figure 2)

¹Disk of dense growth below the soft agar surface (Dinger's disk) [Czekalowski, J. W., J. W. McLeod and J. Rodican. "The Growth and Respiration of *Leptospira* in Solid or Semi-Solid Media with Special Reference to Dinger's Phenomenon." <u>Br. J. Exp. Pathol.</u> 34 (1953): 588-595.] was evident after 20 days at 30°C in an aerobic atmosphere in EMJH semisolid agar (0.15%).

³Determined after 20 days at 30°C in an aerobic atmosphere in EMJH semisolid agar (0.15%) 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.



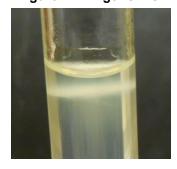
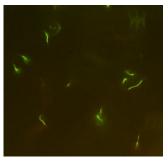


Figure 2: LIVE/DEAD® BacLight™ Bacterial Viability



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²Also consistent with other *Leptospira* species



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