

Certificate of Analysis for NR-22287

Leptospira kirschneri, Strain 1051 (Serovar Bim)

Catalog No. NR-22287

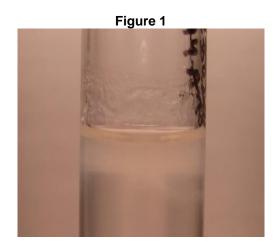
Product Description: Leptospira kirschneri (L. kirschneri), strain 1051 (serovar Bim) was isolated from the kidney of a stray afebrile dog in Barbados. The isolate was obtained from the Centers for Disease Control.

Lot¹: 62380298 Manufacturing Date: 27MAR2014

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology	Spirochete	Spirochete
Colony morphology	Growth below the soft agar surface (Dinger's disk)	Growth below the soft agar surface (Dinger's disk) ² (Figure 1)
Motility (wet mount)	Motile	Motile
Genotypic Analysis Sequencing of 16S ribosomal RNA gene (1230 base pairs)	Consistent with L. kirschneri	Consistent with <i>L. kirschneri</i> ^{3,4}
Purity (post-freeze) ⁵	Growth consistent with L. kirschneri	Growth consistent with L. kirschneri
Viability (post-vialing)		
Visual observation LIVE/DEAD [®] <i>Bac</i> Light [™] Bacterial Viability ⁶	Growth Green fluorescence visible	Growth ² Green fluorescence visible ⁶

The deposited material was grown in Ellinghausen-McCullough-Johnson-Harrison (EMJH) semisolid agar (0.15%) for 47 days at 30°C in an aerobic atmosphere. EMJH containing 20% glycerol was added to an equal volume of culture and the resulting mixture was vialed and frozen. NR-22287 was produced by inoculation of the frozen subculture into EMJH semisolid agar (0.15%) and incubated for 22 days at 30°C in an aerobic atmosphere. The material from the second growth was passaged in EMJH semisolid agar (0.15%) for 22 days at 30°C in an aerobic atmosphere to produce this lot.

⁶Determined after 17 days incubation under cultivation conditions with LIVE/DEAD[®] BacLight[™] Bacterial Viability Kit, 100x 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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²Disk 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 17 days at 30°C in EMJH semisolid agar (0.15%).

³Also consistent with other *Leptospira* species

⁴≥ 99.9 % identical to *L. kirschneri*, strain 1051 (GenBank: AHML02000027)

⁵Purity of this lot was assessed for 17 days on Tryptic Soy agar with 5% defibrinated sheep blood at 37°C in an aerobic atmosphere with 5% CO₂.



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Date: 29 AUG 2014

Signature:

Title:

Technical Manager, BEI Authentication or designee

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