

Certificate of Analysis for NR-22250

Leptospira wolffii, Strain Korat-H2T (Serovar Korat)

Catalog No. NR-22250

Product Description: Leptospira wolffii (L. wolffii), strain Korat-H2T (serovar Korat) was isolated in 2007 from the urine of an adult male in Nakomrachasima Province, Thailand.

Lot¹: 62385762 Manufacturing Date: 13MAR2014

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology	Gram-negative spirochetes	Gram-negative spirochetes
Colony morphology	Growth below the soft agar surface (Dinger's disk)	Growth below the soft agar surface (Dinger's disk) ²
Motility (wet mount)	Report results	Motile
Genotypic Analysis Sequencing of 16S ribosomal RNA gene (~ 1450 base pairs)	Consistent with L. wolffii	Consistent with L. wolffii
Viability (post-vialing) Visual observation LIVE/DEAD [®] BacLight [™] Bacterial Viability	Growth Green fluorescence visible	Growth ² Green fluorescence visible ³

NR-22250 was produced from a frozen subculture of the deposited material. The subculture was cultivated in Ellinghausen-McCullough-Johnson-Harrison (EMJH) semisolid agar (0.15%) for 7 days at 30°C in an aerobic atmosphere. The material from the initial growth was passaged once in EMJH semisolid agar (0.15%) for 7 days at 30°C in an aerobic atmosphere to produce this lot. Purity of this lot was assessed for 7 days on Tryptic Soy agar with 5% defibrinated sheep blood at 37°C in an aerobic atmosphere.

Date: 08 MAY 2014 Signature:

Title: Technical Manager, BEI Authentication or designee

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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 7 days at 30°C in EMJH semisolid agar (0.15%).

³Determined after 7 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.