

Certificate of Analysis for NR-22254

Leptospira kmetyi, Strain Bejo-Iso9T (Serovar Malaysia)

Catalog No. NR-22254

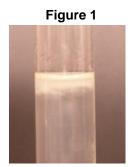
Product Description: Leptospira kmetyi (L. kmetyi), strain Bejo-Iso9T (serovar Malaysia) is a pathogenic strain isolated from a soil sample collected in Johor, Malaysia.

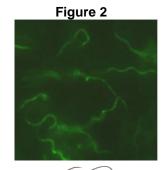
Lot¹: 62380292 Manufacturing Date: 25MAR2014

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 (~ 1450 base pairs)	Consistent with <i>L. kmetyi</i>	Consistent with L. kmetyi ^{3,4}
Viability (post-vialing) Visual observation LIVE/DEAD [®] BacLight [™] Bacterial Viability ⁵	Growth Green fluorescence visible	Growth ² Green fluorescence visible (Figure 2) ⁵

The deposited material was inoculated into Ellinghausen-McCullough-Johnson-Harrison (EMJH) semisolid agar (0.15%) and grown 17 days at 30°C in an aerobic atmosphere, and the resulting growth was vialed and frozen. NR-22254 was produced by inoculation of the frozen subculture into EMJH semisolid agar (0.15%) and incubated for 12 days at 30°C in an aerobic atmosphere. The material from the second growth was passaged in EMJH semisolid agar (0.15%) for 13 days at 30°C in an aerobic atmosphere to produce this lot. Purity of this lot was assessed for 8 days on Tryptic Soy agar with 5% defibrinated sheep blood at 37°C in an aerobic atmosphere with 5% CO₂.

⁵Determined after 65 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.





Date: 26 JUN 2014

Signature:

Title: Technical Manager, BEI Authentication or designee

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² Disk 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 13 days at 30°C in EMJH semisolid agar (0.15%).

³Also consistent with other *Leptospira* species

⁴≥ 99% identical to *L. kmetyi*, strain Bejo-Iso9T (GenBank: AHMP02000003.1)