

Certificate of Analysis for NR-19818

Leptospira interrogans, Strain M776, fur Mutant (Serovar Manilae)

Catalog No. NR-19818

Product Description: Leptospira interrogans (L. interrogans), strain M776 (serovar Manilae) is a transposon mutant of wild-type strain L495 created by disruption of the *fur* gene (*la1857*), which encodes the ferric uptake regulator protein Fur, in strain L495.

Lot¹: 59581725 Manufacturing Date: 06JAN2011

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis Colony morphology	Growth below the soft agar surface (Dinger's disk)	Growth below the soft agar surface (Dinger's disk) ²
Genotypic Analysis Sequencing of 16S ribosomal RNA gene (~ 1360 base pairs)	Consistent with <i>L. interrogans</i>	Consistent with <i>L. interrogans</i> ³
Viability (post-vialing) Visual observation LIVE/DEAD [®] BacLight [™] Bacterial Viability	Growth Green fluorescence visible	Growth ² Green fluorescence visible ⁴

¹L. interrogans, strain M776 (serovar Manilae) was deposited by Ben Adler, Professor of Microbiology, Monash University, Clayton, Victoria, Australia. The deposited material was inoculated into EMJH semisolid agar (0.15%) and incubated for 21 days at 30°C in an aerobic atmosphere. The material from the initial growth was passaged twice in EMJH semisolid agar (0.15%) for 18 days and 17 days, respectively, at 30°C in an aerobic atmosphere to produce this lot.

Date: 31 JAN 2013 Signature: (

Title: Technical Manager, BEI Authentication or designee

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

³Also consistent with other *Leptospira* species

⁴Determined after 15 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.