b|e|i RESOURCES

SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-19820

Leptospira interrogans, Strain M1352, LPS Mutant (Serovar Manilae)

Catalog No. NR-19820

Product Description: *Leptospira interrogans* (*L. interrogans*), strain M1352 (serovar Manilae) is a transposon mutant of wild-type strain L495 created by disruption of a gene of unknown function, *Lman_1408*, located downstream of a putative sugar pyridoxal-phosphate-dependent aminotransferase and 208 base pairs upstream of a putative *rmlC* (dTDP-4-dehydrorhamnose 3,5-epimerase), within the LPS biosynthesis locus of strain L495.

Lot[1]: 59581727 **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 *L. interrogans*	Consistent with *L. interrogans*[3]
Viability (post-vialing) Visual observation LIVE/DEAD® *Bac*Light™ Bacterial Viability	 Growth Green fluorescence visible	 Growth[2] Green fluorescence visible[4]

[1] *L. interrogans*, strain M1352 (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.

[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." Br. J. Exp. Pathol. 34 (1953): 588-595.) was evident after 15 days at 30°C in EMJH semisolid agar (0.15%).

[3] Also consistent with other *Leptospira* species

[4] Determined after 15 days incubation under cultivation conditions with LIVE/DEAD® *Bac*Light™ 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: 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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