Certificate of Analysis for NR-49806

Escherichia coli K-12, Strain IM08B

Catalog No. NR-49806

Product Description: *Escherichia coli* (*E. coli*) K-12, strain IM08B contains the *hsdM* (methylase) and *hsdS* (specificity) genes from *Staphylococcus aureus* NRS384 clonal complex 8 (CC8). This insertion mutant was produced in *E. coli* K-12, strain DC10B (Δ*dcm)*. *E. coli* K-12, strain IM08B was deposited as resistant to streptomycin.

Lot[1]: 63849802 **Manufacturing Date: 18NOV2015**

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology	Gram-negative rods	Gram-negative rods
Colony morphology[2]	Report results	Circular, low convex, entire, smooth and gray (Figure 1)
Motility (wet mount)	Report results	Motile
VITEK® MS (MALDI-TOF)	Consistent with *E. coli*	*E. coli* (99.9%)
Genotypic Analysis		
Sequencing of 16S ribosomal RNA gene (~ 1480 base pairs)	≥ 99% sequence identity to *E. coli* K-12 strain (GenBank: NZ_CP014225.1)	99.5% sequence identity to *E. coli* K-12 strain (GenBank: NZ_CP014225.1)
Riboprinter® Microbial Characterization System	≥ 85% *E. coli*	97% *E. coli*
Analysis of *hsdMS* by PCR Assay[3]		
hsdM2 and *hsdS2* (CC8)	~ 3400 base pair amplicon	~ 3400 base pair amplicon
hsdS1 (CC8)	~ 1770 base pair amplicon	~ 1770 base pair amplicon
Analysis of *hsdMS* by Sequence Analysis[3]		
hsdM2 and *hsdS2* (~ 1710 base pairs)	Consistent with depositor sequence	Consistent with depositor sequence
hsdS1 (~ 1390 base pairs)	Consistent with depositor sequence	Consistent with depositor sequence
Confirmation of Streptomycin Resistance[2]	Growth	Growth
Purity (post-freeze)[4]	Growth consistent with expected colony morphology	Growth consistent with expected colony morphology
Viability (post-freeze)[2]	Growth	Growth

[1]NR-49806 was produced by inoculation of the deposited material into Tryptic Soy broth with 25 µg/mL streptomycin and grown for 1 day at 37°C in an aerobic atmosphere. Broth inoculum was added to Tryptic Soy agar with 25 µg/mL streptomycin kolles, which were grown for 1 day at 37°C in an aerobic atmosphere to produce this lot.

[2]1 day at 37°C in an aerobic atmosphere on Tryptic Soy agar with 25 µg/mL streptomycin

[3]PCR primers used for amplification were IM434 forward primer 5'-ACTTTCTTTAAGGCTTAGAGTCAAGC-3', IM435 reverse primer 5'-TTTAACGCCACGTTCACTCTTTGC-3', 179 forward primer 5'-CGGCCATTTATACAGGAAAAGCCTA-3' and 180 reverse primer 5'-GTTACCTTCTCTATAGAGAGTGGTG-3'. For additional information, refer to Monk, I., et al. "Complete Bypass of Restriction Systems for Major *Staphylococcus aureus* Lineages." MBio. 26 (2015): e00308-15. PubMed: 26015493.

[4]Purity of this lot was assessed for 7 days at 37°C in an aerobic atmosphere on Tryptic Soy agar with 5% defibrinated sheep blood.

Figure 1: Colony Morphology

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Date: 15 APR 2016 **Signature:** 

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