

Certificate of Analysis for NR-49808

Escherichia coli K-12, Strain IM93B

Catalog No. NR-49808

Product Description: Escherichia coli (E. coli) K-12, strain IM93B contains the hsdM (methylase) and hsdS (specificity) genes from Staphylococcus aureus JKD6159 sequence type 93 (ST93). This insertion mutant was produced in E. coli K-12, strain DC10B (∆dcm). E. coli K-12, strain IM93B was deposited as resistant to streptomycin.

Lot¹: 70015549 Manufacturing Date: 13JUN2018

TEST	SPECIFICATIONS	RESULTS
	or contextions	I I I I I I I I I I I I I I I I I I I
Phenotypic Analysis Cellular morphology Colony morphology ²	Gram-negative rods Report results	Gram-negative rods Circular, convex, entire, smooth and
Motility (wet mount) VITEK® MS (MALDI-TOF)	Report results E. coli	cream (Figure 1) Motile E. coli (99.9%)
Genotypic Analysis Sequencing of 16S ribosomal RNA gene (~ 1480 base pairs)	≥ 99% sequence identity to <i>E. coli</i> K-12 strain (GenBank: NZ_CP014225.1)	99.8% sequence identity to E. coli K-12 strain (GenBank: NZ_CP014225.1) ³
Analysis of hsdMS by PCR Assay ^{4,5} hsdM2, hsdS2 and hsdS1 (ST93) hsdM3 and hsdS3 (ST93)	~ 3200 base pair amplicon ~ 4600 base pair amplicon	~ 3200 base pair amplicon ~ 4600 base pair amplicon
Analysis of hsdMS by Sequence Analysis ^{4,5} hsdM2, hsdS2 and hsdS1 (~ 740 base pairs) hsdM3 and hsdS3 (~ 1010 base pairs)	Consistent with depositor sequence Consistent with depositor sequence	Consistent with depositor sequence Consistent with depositor sequence
Confirmation of Streptomycin Resistance ²	Growth	Growth
Purity (post-freeze) Tryptic Soy agar ⁶ Tryptic Soy agar with 5% defibrinated sheep blood ⁷	Growth consistent with expected colony morphology Growth consistent with expected colony morphology	Growth consistent with expected colony morphology Growth consistent with expected colony morphology
Viability (post-freeze) ²	Growth	Growth

NR-49808 lot 70015549 was produced by the inoculation of BEI Resources NRS-49808 lot 63849810 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.

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²1 day at 37°C in an aerobic atmosphere on Tryptic Soy agar with 25 μg/mL streptomycin

³Also consistent with Shigella and other Escherichia species

⁴These tests were performed on the previous lot of extracted material (NRS-49808 lot 63849810).

⁵PCR 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'-GTTACCTTCTATAGAGAGAGTGGTG-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.

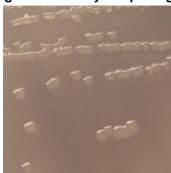
⁶Purity of this lot was assessed for 7 days at 37°C in an aerobic atmosphere with 5% CO₂.

⁷Purity of this lot was assessed for 7 days at 37°C in an aerobic atmosphere.



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Figure 1: Colony Morphology



/Heather Couch/ Heather Couch

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Program Manager or designee, ATCC Federal Solutions

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