

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 (Δdcm). E. coli K-12, strain IM93B was deposited as resistant to streptomycin.

Lot¹: 63849809 Manufacturing Date: 19NOV2015

| TEST | SPECIFICATIONS | RESULTS |
|--|---|---|
| Phenotypic Analysis | | |
| Cellular morphology | Gram-negative rods | Gram-negative rods |
| Colony morphology ² | Report results | Circular, slight peaked, entire, |
| | | smooth and cream (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 | ≥ 99% sequence identity to <i>E. coli</i> | 99.5% sequence identity to E. coli |
| (~ 1480 base pairs) | K-12 strain (GenBank: | K-12 strain (GenBank: |
| @ | NZ_CP014225.1) | NZ_CP014225.1) |
| Riboprinter® Microbial Characterization System | ≥ 85% <i>E. coli</i> | 95% E. coli |
| Analysis of <i>hsdMS</i> by PCR Assay ³ | | |
| hsdM2, hsdS2, hsdS1 (ST93) | ~ 3200 base pair amplicon | ~ 3200 base pair amplicon |
| hsdM3 and hsdS3 (ST93) | ~ 4600 base pair amplicon | ~ 4600 base pair amplicon |
| Analysis of <i>hsdMS</i> by Sequence Analysis ³ | | |
| hsdM2 and hsdS2 (~ 740 base pairs) | Consistent with depositor sequence | Consistent with depositor sequence |
| hsdS1, hsdM3 and hsdS3 (~ 1010 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 |

NR-49808 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.

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

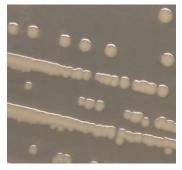


Figure 1: Colony Morphology

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

³PCR 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.



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Date: 15 APR 2016

Signature:

BEI Resources Authentication

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