

Certificate of Analysis for NR-49807

Escherichia coli K-12, Strain IM30B

Catalog No. NR-49807

Product Description: Escherichia coli (E. coli) K-12, strain IM30B contains the hsdM (methylase) and hsdS (specificity) genes from Staphylococcus aureus MRSA252 clonal complex 30 (CC30). This insertion mutant was produced in E. coli K-12, strain DC10B (Δdcm). E. coli K-12, strain IM30B was deposited as resistant to streptomycin.

Lot¹: 63849808 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, translucent, 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>	99.8% sequence identity to <i>E. coli</i>
(~ 1410 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>	96% E. coli
Analysis of hsdMS by PCR Assay ³		
hsdM2 and hsdS2 (CC30)	~ 3400 base pair amplicon	~ 3400 base pair amplicon
hsdS1 (CC30)	~ 1800 base pair amplicon	~ 1800 base pair amplicon
Analysis of <i>hsdMS</i> by Sequence Analysis ³		
hsdM2 and hsdS2 (~ 1650 base pairs)	Consistent with depositor sequence	Consistent with depositor sequence
hsdS1 (~ 880 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-49807 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.





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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: (

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