SUPPORTING INFECTIOUS DISEASE RESEARCH

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: 70015548

Manufacturing Date: 13JUN2018

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis Cellular morphology Colony morphology ²	Gram-negative rods Report results	Gram-negative rods Circular, convex, entire, smooth and cream (Figure 1)
Motility (wet mount) VITEK [®] MS (MALDI-TOF)	Report results <i>E. coli</i>	Motile <i>E. coli</i> (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 <i>E. coli</i> K-12 strain (GenBank: NZ_CP014225.1) ³
Analysis of hsdMS by PCR Assay ^{4,5} hsdM2 and hsdS2 (CC30) hsdS1 (CC30)	~ 3400 base pair amplicon ~ 1800 base pair amplicon	~ 3400 base pair amplicon ~ 1800 base pair amplicon
Analysis of hsdMS by Sequence Analysis ^{4,5} hsdM2 and hsdS2 (~ 1650 base pairs) hsdS1 (~ 880 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

¹NR-49807 lot 70015548 was produced by the inoculation of BEI Resources NRS-49807 lot 63849807 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.

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 (NR-49807 lot 63849808).

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'-GTTACCTTCTCATAGAGAGTGGTG-3'. For additional information, refer to Monk, I., et al. "Complete Bypass of Restriction Systems for Major *Staphylococcus aureus* Lineages." <u>mBio</u> 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.

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Certificate of Analysis for NR-49807

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Figure 1: Colony Morphology



/Heather Couch/ Heather Couch

29 AUG 2018

Program Manager or designee, ATCC Federal Solutions

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