

**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 (CC). This insertion mutant was produced in *E. coli* K-12, strain DC10B via recombination-mediated genetic engineering (recombineering). *E. coli* K-12, strain IM30B was deposited as resistant to streptomycin. NR-49807 was produced by the inoculation of BEI Resources seed lot 63849807 into Tryptic Soy broth containing 25 µg/mL streptomycin and grown for 1 day at 37°C in an aerobic atmosphere. Broth inoculum was added to Tryptic Soy agar containing 25 µg/mL streptomycin kolles, which were grown for 1 day at 37°C in an aerobic atmosphere to produce this lot. Quality control testing was completed under propagation conditions unless otherwise noted.

**Lot: 70056754**

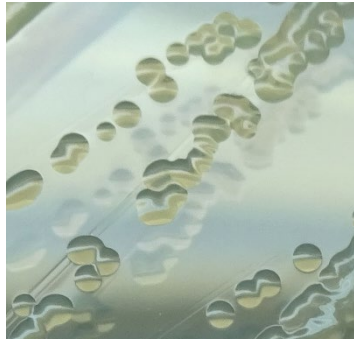
**Manufacturing Date: 09NOV2022**

TEST	SPECIFICATIONS	RESULTS
<b>Phenotypic Analysis</b> Cellular morphology Colony morphology  Motility (wet mount) VITEK® MS (MALDI-TOF)	Gram-negative rods Report results  Report results <i>Escherichia coli</i>	Gram-negative rods Circular, convex, entire, smooth and cream (Figure 1)  Motile <i>Escherichia coli</i> (99.9%)
<b>Genotypic Analysis</b> Sequencing of 16S ribosomal RNA gene (~ 1480 base pairs)	≥ 99% sequence identity to <i>E. coli</i> , strain K-12 (GenBank: CP014225.1)	99.7% sequence identity to <i>E. coli</i> , strain K-12 (GenBank: CP014225.1)
<b>Analysis of <i>hsdMS</i> by PCR Assay<sup>1,2</sup></b> <i>hsdM2</i> and <i>hsdS2</i> (CC30) <i>hsdS1</i> (CC30)	~ 3400 base pair amplicon ~ 1800 base pair amplicon	~ 3400 base pair amplicon ~ 1800 base pair amplicon
<b>Analysis of <i>hsdMS</i> by Sequence Analysis<sup>1,2</sup></b> <i>hsdM2</i> and <i>hsdS2</i> (~ 1650 base pairs) <i>hsdS1</i> (~ 880 base pairs)	Consistent with depositor sequence Consistent with depositor sequence	Consistent with depositor sequence Consistent with depositor sequence
<b>Confirmation of Streptomycin Resistance</b>	Growth	Growth
<b>Purity</b> 7 days at 37°C in an aerobic atmosphere with and without 5% CO <sub>2</sub> on Tryptic Soy agar with 5% defibrinated sheep blood	Consistent with expected colony morphology	Consistent with expected colony morphology
<b>Viability</b>	Growth	Growth

<sup>1</sup>Performed on the previous lot of extracted material (NR-49807 lot 63849808).

<sup>2</sup>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'-GTTACCTTCTCTATAGAGTGGTG-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.

Figure 1: Colony Morphology



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