

Certificate of Analysis for NR-47465

Staphylococcus aureus subsp. aureus, Strain JE2, Transposon Mutant NE922 (SAUSA300_0124)

Catalog No. NR-47465

Product Description: Staphylococcus aureus (S. aureus) subsp. aureus, transposon mutant NE922 was derived from S. aureus subsp. aureus, strain JE2. Mutagenesis occurred through the use of the mariner-based transposon bursa aurealis resulting in an erythromycin-resistant deletion strain of JE2. S. aureus subsp. aureus, transposon mutant NE922 was created by disruption of SAUSA300_0124, which encodes for a pyridoxal-phosphate dependent enzyme superfamily protein. This protein has been identified as SbnG, a citrate synthase. SbnG is expressed under iron-limiting conditions, and provides the precursor citrate for staphyloferrin B biosynthesis. Staphyloferrin B is a virulence-associated siderophore necessary to scavenge iron within the host. Strain JE2 is a plasmid-cured derivative of strain LAC that was isolated in 2002 from a skin and soft tissue infection of an inmate in the Los Angeles County Jail in California, USA.

Lot¹: 70012417 Manufacturing Date: 31JAN2018

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis Cellular morphology Colony morphology ² Motility (wet mount)	Gram-positive cocci Report results Report results	Gram-positive cocci Circular, low convex, entire, smooth and cream (Figure 1) Non-motile
Confirmation of Transposon Insertion ³	Resistant to erythromycin	Resistant to erythromycin
Purity (post-freeze) ⁴	Consistent with expected colony morphology	Consistent with expected colony morphology
Viability (post-freeze) ²	Growth	Growth

¹NR-47465 was produced by inoculation of the deposited material into Tryptic Soy broth with 5 μg/mL erythromycin and incubated for 1 day at 37°C in an aerobic atmosphere. Broth inoculum was added to Tryptic Soy agar with 5 μg/mL erythromycin kolles which were grown 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 with 5% CO₂ 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 5 µg/mL erythromycin

³Prior to initiating work, it is recommended that the presence and location of the transposon is confirmed. Gene specific primers should be paired with either the "Upstream" primer (5'-CTCGATTCTATTAACAAGGG-3') for transposons in the "plus" orientation or the "Buster" primer (5'-GCTTTTTCTAAATGTTTTTTAAGTAAATCAAGTAC-3') for transposons in the "minus" orientation. For additional information, refer to Fey, P. D., et al. "A Genetic Resource for Rapid and Comprehensive Phenotype Screening of Nonessential *Staphylococcus aureus* Genes." mBio 4 (2013): e00537-12. PubMed: 23404398.



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28 MAR 2018

Program Manager or designee, ATCC Federal Solutions

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