

Certificate of Analysis for NR-48060

Staphylococcus aureus subsp. aureus, Strain JE2, Transposon Mutant NE1518 (SAUSA300_0861)

Catalog No. NR-48060

Product Description: Staphylococcus aureus (S. aureus) subsp. aureus, transposon mutant NE1518 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 NE1518 was created by disruption of gudB, a NAD-specific glutamate dehydrogenase that is important for growth when glucose supply is limited. 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: 70011331 Manufacturing Date: 08DEC2017

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology	Gram-positive cocci	Gram-positive cocci
Colony morphology ²	Report results	Circular, convex, entire, smooth and cream (Figure 1)
Motility (wet mount)	Report results	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 ⁵
Viability (post-freeze) ²	Growth	Growth

¹NR-48060 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.

Figure 1: Colony Morphology



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www.beiresources.org

E-mail: contact@beiresources.org

Tel: 800-359-7370 Fax: 703-365-2898

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." <u>mBio</u> 4 (2013): e00537-12. PubMed: 23404398.

⁴Purity of this lot was assessed for 7 days at 37°C in an aerobic atmosphere with 5% CO₂ on Tryptic Soy agar.

⁵Two colony types were observed after 7 days. Colony type 1 was circular, convex, entire, smooth and cream, whereas colony type 2 was irregular, spreading and white. Plating of the individual colony types showed that colony type 2 reverted to colony type 1. The 16S ribosomal RNA gene of each colony type was sequenced and found to have 100% sequence identity with the other colony type and consistent with *S. aureus* subsp. *aureus*.



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09 FEB 2018

Program Manager or designee, ATCC Federal Solutions

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