

Certificate of Analysis for NR-46691

Staphylococcus aureus subsp. aureus, Strain JE2, Transposon Mutant NE148 (SAUSA300_0283)

Catalog No. NR-46691

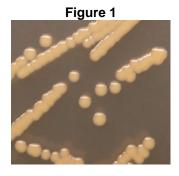
Product Description: Staphylococcus aureus (S. aureus) subsp. aureus, transposon mutant NE148 was derived from S. aureus subsp. aureus, strain JE2. Mutagenesis occurred through the use of the mariner-based transposon bursa aurealis resulting in an erythromycin-resistant deletion strain of JE2. S. aureus subsp. aureus, transposon mutant NE148 was created by disruption of SAUSA300_0283 encoding for the ESAT-6 secretion system (Ess) pathway protein EssC that contributes to the virulence of S. aureus through production and secretion of EsxA and EsxB. 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: 63323976 Manufacturing Date: 25FEB2015

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

¹NR-46691 was produced by inoculation of the deposited material into Tryptic Soy broth with 5 μg/mL erythromycin and incubated for 24 hours at 37°C in an aerobic atmosphere. Broth inoculum was added to Tryptic Soy agar with 5 μg/mL erythromycin kolles which were grown 20 hours 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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²24 hours 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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Date: 10 APR 2015

Signature: (

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