

## **Certificate of Analysis for NR-46544**

Staphylococcus aureus subsp. aureus, Strain JE2, Transposon Mutant NE1 (SAUSA300\_1327)

Catalog No. NR-46544

**Product Description:** *Staphylococcus aureus (S. aureus)* subsp. *aureus*, transposon mutant NE1 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 NE1 was created by disruption of SAUSA300\_1327, which encodes for a hyperosmolarity resistance protein, Ebh, involved in tolerance to transient hyperosmotic pressure, cell size determination, resistance to methicillin and virulence. 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.

Lot<sup>1</sup>: 70018983 Manufacturing Date: 14SEP2018

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

<sup>&</sup>lt;sup>1</sup>NR-46544 lot 70018983 was produced by inoculation of BEI Resources NRS-46544 lot 63025339 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.

<sup>&</sup>lt;sup>4</sup>Purity of this lot was assessed for 7 days at 37°C in an aerobic atmosphere on Tryptic Soy agar with 5% defibrinated sheep blood.



Figure 1: Colony Morphology

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<sup>&</sup>lt;sup>2</sup>1 day at 37°C in an aerobic atmosphere on Tryptic Soy agar with 5 μg/mL erythromycin

<sup>&</sup>lt;sup>3</sup>Prior 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.



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Program Manager or designee, ATCC Federal Solutions

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