Certificate of Analysis for NR-46545

Staphylococcus aureus subsp. *aureus*, Strain JE2, Transposon Mutant NE2 (SAUSA300_1509)

Catalog No. NR-46545

Product Description: *Staphylococcus aureus* (*S. aureus*) subsp. *aureus,* transposon mutant NE2 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 NE2 was created by disruption of SAUSA300_1509*,* which encodes for a rhomboid family peptidase that may be involved in signaling events at the plasma membrane. 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[1]: 63043376 **Manufacturing Date: 30OCT2014**

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology	Gram-positive cocci	Gram-positive cocci
Colony morphology[2,3]	Report results	Circular, convex, entire, smooth and cream (Figure 1)
Motility (wet mount)	Report results	Non-motile
Confirmation of Transposon Insertion[4]	Resistant to erythromycin	Resistant to erythromycin
Purity (post-freeze)[5]	Growth consistent with *S. aureus*	Growth consistent with *S. aureus*
Viability (post-freeze)[2]	Growth	Growth

[1]NR-46545 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 24 hours at 37°C in an aerobic atmosphere to produce this lot.

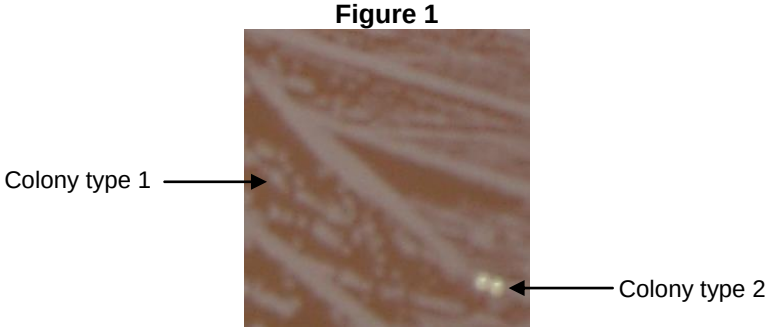
[2]22 hours at 37°C in an aerobic atmosphere on Tryptic Soy agar with 5 µg/mL erythromycin

[3]After 7 days of incubation, a second colony type was observed that was circular, convex, entire, smooth and yellow. Plating of this second colony type showed that it reverted to the initial colony type. Cells from the second colony type were subjected to VITEK® MS (MALDI-TOF) analysis and identified as *S. aureus*. Since NR-46545 is a transposon mutant and is expected produce a single colony type, colony purification of this item is highly recommended.

[4]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." MBio 4 (2013): e00537-12. PubMed: 23404398.

[5]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



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Date: 12 DEC 2014 **Signature:** 

Title: Technical Manager, BEI Authentication or designee

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC®'s knowledge.

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