Certificate of Analysis for NR-47875

Staphylococcus aureus subsp. *aureus*, Strain JE2, Transposon Mutant NE1333 (SAUSA300_1542)

Catalog No. NR-47875

Product Description: *Staphylococcus aureus* (*S. aureus*) subsp. *aureus,* transposon mutant NE1333 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 NE1333 was created by disruption of *hrcA*, which encodes for a heat-inducible transcriptional regulator involved in repression of *S. aureus* class I heat shock genes such as *dnaK* and *groESL* operons. 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]: 70004180 **Manufacturing Date: 31MAR2017**

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

[1]NR-47875 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.

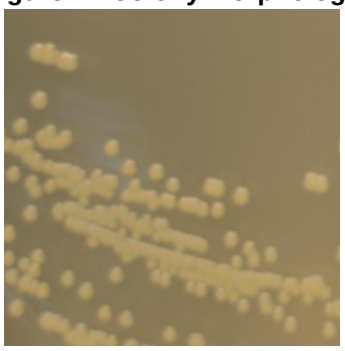
[2]1 day at 37°C in an aerobic atmosphere on Tryptic Soy agar with 5 µg/mL erythromycin.

[3]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.

[4]Purity of this lot was assessed for 7 days at 37°C in an aerobic atmosphere with 5% CO_2 on Tryptic Soy agar with 5% defibrinated sheep blood.

[5]A second colony type consistent with *S. aureus* was observed after an extended incubation on Tryptic Soy agar with 5% defibrinated sheep blood. VITEK® MS (MALDI-TOF) analysis identified cells from both colony types as *S. aureus*. Since NR-47875 is a transposon mutant and is expected to produce a single colony type, colony purification of this item is highly recommended.

Figure 1: Colony Morphology



BEI Resources
www.beiresources.org
E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898

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Date: 08 MAY 2017 **Signature:** 

BEI Resources Authentication

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BEI Resources
www.beiresources.org

E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898