Certificate of Analysis for NR-47717

Staphylococcus aureus subsp. *aureus*, Strain JE2, Transposon Mutant NE1174 (SAUSA300_2411)

Catalog No. NR-47717

Product Description: *Staphylococcus aureus (S. aureus)* subsp. *aureus,* transposon mutant NE1174 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 NE1174 was created by disruption of *opp-1A*, which encodes for a substrate binding protein, CntA, that is part of the transition-metal acquisition system Cnt. Cnt contributes to *S. aureus* virulence by utilizing the metallophore staphylopine to uptake zinc as well as other transition metals to overcome nutritional immunity imposed by host-mediated restriction of essential nutrients. 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]: 70012383 **Manufacturing Date: 14FEB2018**

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
Viability (post-freeze)[2]	Growth	Growth

[1]NR-47717 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.

Figure 1: Colony Morphology

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

Certificate of Analysis for NR-47717

Heather Couch

Digitally signed by Heather Couch
Date: 2018.05.25 09:09:59 -04'00'

25 MAY 2018

Program Manager or designee, ATCC Federal Solutions

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.

ATCC® is a trademark of the American Type Culture Collection.
You are authorized to use this product for research use only. It is not intended for human use.

Support Provided by NIAID

BEI Resources
www.beiresources.org

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