SUPPORTING INFECTIOUS DISEASE RESEARCH

Antimicrobial Resistance Panel 13: *Pseudomonas aeruginosa* Fluoroquinolone Resistance Pathway Mutants

Catalog No. NR-55652

Product Description:

NR-55652 consists of a 2-member panel of *Pseudomonas aeruginosa* (*P. aeruginosa*) PAO1 mutant strains. NB52023 is an efflux deficient mutant lacking *mexXY* operon genes: *mexB* and *mexX* ($\Delta mexB \Delta mexX$). NB52023-CDK0006 is derived from NB52023 by site-directed mutagenesis and contains mutations resulting in amino acid substitutions in DNA gyrase [*gyrA* (T831)] and topoisomerase IV [*parC* (S87L)].

NR-51969 and NR-51866 were produced by inoculating the deposited material into Tryptic Soy broth and grown for 1 day at 37°C in an aerobic atmosphere. Broth inoculum was added to Tryptic Soy agar kolles, which were grown for 1 day at 37°C in an aerobic atmosphere to produce lots 70046521 and 70048187, respectively.

Quality control testing was completed under propagation conditions unless otherwise noted.

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COMPONENT NUMBER	STRAIN	DESCRIPTION	LOT NUMBER	MANUFACTURING DATE		
NR-51969	<i>P. aeruginosa</i> , strain NB52023	$\Delta mex B \Delta mex XY$	70046521	13AUG2021		
NR-51866	<i>P. aeruginosa</i> , strain NB52023-CDK0006	ΔmexB ΔmexX gyrA (T83I) parC (S87L)	70048187	210CT2021		

Table 1: Kit Components

Table 2: P. aeruginosa, Strain NB52023 (NR-51969)

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology	Gram-negative rods	Gram-negative rods
Colony morphology	Report results	Irregular, convex, undulate, smooth and cream
Motility (wet mount)	Report results	Motile
VITEK [®] MS (MALDI-TOF)	P. aeruginosa	P. aeruginosa (99.9%)
Antibiotic Susceptibility Profile		
Etest [®] antibiotic test strips		
1 day at 35°C in an aerobic atmosphere on		
Mueller Hinton agar		
Chloramphenicol	Report results	> 1µg/mL
Ofloxacin	Report results	0.125 μg/mL
Genotypic Analysis		
Digital DNA-DNA hybridization (dDDH) ¹	≥ 70% for species identification	P. aeruginosa (95.2%)
Deletion of <i>mexB</i> and <i>mexX</i>	Deletions confirmed	Pending
Purity (post-freeze)	Growth consistent with expected	Growth consistent with expected
8 days at 37°C in an aerobic atmosphere with	colony morphology	colony morphology
and without 5%CO ₂ on Tryptic Soy agar		
Viability (post-freeze)	Growth	Growth

¹Relatedness between bacterial strains has traditionally been determined using DDH. For additional information, refer to Auch, A. F., et al. "Digital DNA-DNA Hybridization for Microbial Species Delineation by Means of Genome-to-Genome Sequence Comparison." <u>Stand. Genomic Sci.</u> 2 (2010): 117-134. PubMed: 21304684.

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Certificate of Analysis for NR-55652

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TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology	Gram-negative rods	Gram-negative rods
Colony morphologies ¹	Report results	Colony type 1: Irregular, low convex, undulate, rugose and cream Colony type 2: Circular, low convex, entire, smooth and cream
Motility (wet mount)	Report results	Motile
VITEK [®] MS (MALDI-TOF)	P. aeruginosa	P. aeruginosa (99.9%)
Antibiotic Susceptibility Profile		
BD BBL™ Sensi-Disc™ Susceptibility Test		
Discs		
1 day at 35°C in an aerobic atmosphere on Mueller Hinton agar		
Gatifloxacin	Report results	11-12 mm
Novobiocin	Report results	0 mm
Genotypic Analysis		
Digital DNA-DNA hybridization (dDDH) ²	≥ 70% for species identification	P. aeruginosa (95.2%)
Deletion of <i>mexB</i> and <i>mexX</i>	Deletions confirmed	Pending
Confirmation of gyrA (T83I) parC (S87L) SNPs	SNPs confirmed	Pending
Purity	Growth consistent with expected	Growth consistent with expected
7 days at 37°C in an aerobic atmosphere with and without 5% CO ₂ on Tryptic Soy agar	colony morphology	colony morphology
Viability	Growth	Growth

¹Two colony types were observed. Plating of the individual colony types showed that they reverted to colony type 1. VITEK[®] MS (MALDI-TOF) analysis identified cells from both colony types as *P. aeruginosa*.

²Relatedness between bacterial strains has traditionally been determined using DDH. For additional information, refer to Auch, A. F., et al. "Digital DNA-DNA Hybridization for Microbial Species Delineation by Means of Genome-to-Genome Sequence Comparison." <u>Stand. Genomic Sci.</u> 2 (2010): 117-134. PubMed: 21304684.

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