Certificate of Analysis for NR-55643

Antimicrobial Resistance Panel 4: *Pseudomonas aeruginosa* Elongation Factor G

Catalog No. NR-55643

Product Description:

NR-55643 consists of a 5-member panel of *Pseudomonas aeruginosa (P. aeruginosa)* mutant strains generated by growing *P. aeruginosa,* strains K767 and Z61 (ATCC® 35151™) on agar containing 128 µg per mL of argyrin. Sequencing of the resulting strains identified point mutations in the *fus*A1 gene, resulting in amino acid substitutions in Elongation Factor G (EF-G). *P. aeruginosa*, strains NB52019-CDR0055, NB52019-CDR0054 and NB52019-CDR0056 were derived from *P. aeruginosa,* strain K767. *P. aeruginosa,* strains NB52040-CDA0055 and NB52040-CDA0056 were derived from *P. aeruginosa*, strain Z61.

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Table 1: Kit Components

COMPONENT NUMBER	DESCRIPTION	FusA1 MUTATION	LOT NUMBER	MANUFACTURING DATE
NR-51930	*P. aeruginosa*, strain NB52019-CDR0055	S417L	70044804	27MAY2021
NR-51931	*P. aeruginosa*, strain NB52019-CDR0054	S459F	70044806	26MAY2021
NR-51932	*P. aeruginosa*, strain NB52019-CDR0056	Y683C	70044808	04JUN2021
NR-51934	*P. aeruginosa*, strain NB52040-CDA0055	S417L	70044812	09JUN2021
NR-51935	*P. aeruginosa*, strain NB52040-CDA0056	S459F	70044814	24JUN2021

NR-51930 and NR-51932 were produced by inoculation of the deposited material into Tryptic Soy broth and grown for 2 days 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 70044804 and 70044808, respectively.

NR-51931 was produced by inoculation of 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 lot 70044806.

NR-51934 was produced by inoculation of the deposited material into Tryptic Soy broth and grown for 2 days at 37°C in an aerobic atmosphere. After a 3-day hold at 4°C in an aerobic atmosphere, the growth was passaged on Tryptic Soy agar with 5% defibrinated sheep blood for 1 day at 37°C in an aerobic atmosphere and colonies were suspended in Tryptic Soy broth. This suspension was used to inoculate Tryptic Soy agar kolles, which were grown for 1 day at 37°C in an aerobic atmosphere to produce lot 70044812.

NR-51935 was produced by inoculation of the deposited material onto Tryptic Soy agar and grown for 2 days at 37°C in an aerobic atmosphere, and the resulting subculture was vialed and frozen in Tryptic Soy broth supplemented with 10% glycerol. The frozen subculture was used to inoculate Tryptic Soy broth, which was grown for 1 day at 37°C in an aerobic atmosphere. Broth inoculum was added to Tryptic Soy agar with 5% defibrinated sheep blood kolles and grown for 1 day at 37°C in an aerobic atmosphere to produce lot 70044814.

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

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Table 2: *P. aeruginosa*, Strain NB52019-CDR0055 (NR-51930)

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology	Gram-negative rods	Gram-negative rods
Colony morphology	Report results	Circular, low convex, undulate, smooth and cream (Figure 1a)
Motility (wet mount)	Report results	Motile
VITEK® MS (MALDI-TOF)	*P. aeruginosa*	*P. aeruginosa* (99.9%)
Antibiotic Susceptibility Profile[1]		
Etest® antibiotic test strips 1 day at 35°C in an aerobic atmosphere on Mueller Hinton agar		
Amikacin	Report results	Resistant (64 µg per mL)
Gentamycin	Report results	Resistant (16 µg per mL)
Tobramycin	Report results	Resistant (12 µg per mL)
Genotypic Analysis		
Digital DNA-DNA hybridization (dDDH)[2]	≥ 70% for species identification	*P. aeruginosa* (95.1%)
Detection of reported point mutation	Point mutation present	Pending
Purity 7 days at 37°C in an aerobic atmosphere on Tryptic Soy agar with 5% defibrinated sheep blood agar	Growth consistent with expected colony morphology	Growth consistent with expected colony morphology
Viability	Growth	Growth

Table 3: *P. aeruginosa*, Strain NB52019-CDR0054 (NR-51931)

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology	Gram-negative rods	Gram-negative rods
Colony morphology	Report results	Circular, low convex, undulate, smooth and cream (Figure 1b)
Motility (wet mount)	Report results	Motile
VITEK® MS (MALDI-TOF)	*P. aeruginosa*	*P. aeruginosa* (99.9%)
Antibiotic Susceptibility Profile[1]		
Etest® antibiotic test strips 1 day at 35°C in an aerobic atmosphere on Mueller Hinton agar		
Amikacin	Report results	Sensitive (16 µg per mL)
Gentamycin	Report results	Resistant (12 µg per mL)
Tobramycin	Report results	Sensitive (3-4 µg per mL)
Genotypic Analysis		
Digital DNA-DNA hybridization (dDDH)[2]	≥ 70% for species identification	*P. aeruginosa* (95.2%)
Detection of reported point mutation	Point mutation present	Pending
Purity 7 days at 37°C in an aerobic atmosphere with and without 5% CO_2 on Tryptic Soy agar with 5% defibrinated sheep blood agar	Growth consistent with expected colony morphology	Growth consistent with expected colony morphology
Viability	Growth	Growth

[1]Minimum Inhibitory Concentration (MIC); MIC Interpretation Guideline: CLSI M100-S28 (2018)
[2]Relatedness 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." Stand. Genomic Sci. 2 (2010): 117-134. PubMed: 21304684.

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Table 4: *P. aeruginosa*, Strain NB52019-CDR0056 (NR-51932)

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology	Gram-negative rods	Gram-negative rods
Colony morphology	Report results	Circular, convex, undulate, smooth and cream (Figure 1c)
Motility (wet mount)	Report results	Motile
VITEK® MS (MALDI-TOF)	*P. aeruginosa*	*P. aeruginosa* (99.9%)
Antibiotic Susceptibility Profile[1]		
Etest® antibiotic test strips		
1 day at 35°C in an aerobic atmosphere on Mueller Hinton agar		
Amikacin	Report results	Intermediate (16-24 µg per mL)
Gentamycin	Report results	Intermediate (4-6 µg per mL)
Tobramycin	Report results	Sensitive (3 µg per mL)
Genotypic Analysis		
Digital DNA-DNA hybridization (dDDH)[2]	≥ 70% for species identification	*P. aeruginosa* (95.3%)
Detection of reported point mutation	Point mutation present	Pending
Purity 7 days at 37°C in an aerobic atmosphere on Tryptic Soy agar with 5% defibrinated sheep blood agar	Growth consistent with expected colony morphology	Growth consistent with expected colony morphology
Viability	Growth	Growth

Table 5: *P. aeruginosa*, Strain NB52040-CDA0055 (NR-51934)

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology	Gram-negative rods	Gram-negative rods
Colony morphology	Report results	Circular, low convex, entire, smooth and cream (Figure 1d)
Motility (wet mount)	Report results	Motile
VITEK® MS (MALDI-TOF)	*P. aeruginosa*	*P. aeruginosa* (99.9%)
Antibiotic Susceptibility Profile[1]		
Etest® antibiotic test strips		
1 day at 35°C in an aerobic atmosphere on Mueller Hinton agar		
Amikacin	Report results	Sensitive (2-3 µg per mL)
Gentamycin	Report results	Sensitive (0.75 µg per mL)
Tobramycin	Report results	Sensitive (0.75 µg per mL)
Genotypic Analysis		
Digital DNA-DNA hybridization (dDDH)[2]	≥ 70% for species identification	*P. aeruginosa* (95.2%)
Detection of reported point mutation	Point mutation present	Pending
Purity 7 days at 37°C in an aerobic atmosphere with and without 5% CO_2 on Tryptic Soy agar	Growth consistent with expected colony morphology	Growth consistent with expected colony morphology
Viability	Growth	Growth

[1]Minimum Inhibitory Concentration (MIC); MIC Interpretation Guideline: CLSI M100-S28 (2018)
[2]Relatedness 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." Stand. Genomic Sci. 2 (2010): 117-134. PubMed: 21304684.

Table 6: *P. aeruginosa*, Strain NB52040-CDA0056 (NR-51935)

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology	Gram-negative rods	Gram-negative rods
Colony morphology	Report results	Circular, slight peaked, undulate, smooth and cream
Motility (wet mount)	Report results	Motile
VITEK® MS (MALDI-TOF)	*P. aeruginosa*	*P. aeruginosa* (99.9%)
Antibiotic Susceptibility Profile[1]		
Etest® antibiotic test strips		
1 day at 35°C in an aerobic atmosphere on Mueller Hinton agar		
Amikacin	Report results	Sensitive (3-4 µg per mL)
Gentamycin	Report results	Sensitive (0.75-1.0 µg per mL)
Tobramycin	Report results	Sensitive (0.50-0.75 µg per mL)
Genotypic Analysis		
Digital DNA-DNA hybridization (dDDH)[2]	≥ 70% for species identification	*P. aeruginosa* (95.3%)
Detection of reported point mutation	Point mutation present	Pending
Purity 8 days at 37°C in an aerobic atmosphere with 5% CO_2 on Tryptic Soy agar with 5% defibrinated sheep blood	Growth consistent with expected colony morphology	Growth consistent with expected colony morphology
Viability	Growth	Growth

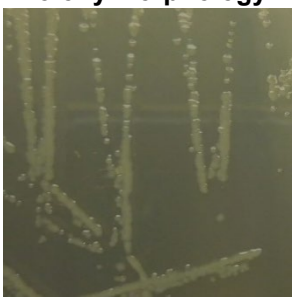
[1]Minimum Inhibitory Concentration (MIC); MIC Interpretation Guideline: CLSI M100-S28 (2018)
[2]Relatedness between bacterial strains has traditionally been determined using DDH. For additional information, refer to Auch, A. F., et al. "Digital DNA-DNA Hybridization for Microbial Species Delineation by Means of Genome-to-Genome Sequence Comparison." Stand. Genomic Sci. 2 (2010): 117-134. PubMed: 21304684.

Figure 1a: NR-51930 Colony Morphology

Figure 1b: NR-51931 Colony Morphology

Figure 1c: NR-51932 Colony Morphology

Figure 1d: NR-51934 Colony Morphology



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