Certificate of Analysis for NR-55641

Antimicrobial Resistance Panel 2: Multiple Species Coenzyme A (CoA-SH) Biosynthesis Pathway

Catalog No. NR-55641

Product Description:

NR-55641 consists of laboratory-generated, efflux deficient mutant strains of *Escherichia coli (E. coli), Haemophilus influenzae (H. influenzae)* and *Klebsiella pneumoniae (K. pneumoniae).*

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Table 1: Kit Components

COMPONENT NUMBER	DESCRIPTION	GENOTYPE	LOT NUMBER	DATE OF MANUFACTURE
NR-51923	*Escherichia coli*, NB27079-CDY0099	Δ*acrB*, Δ*acrD*, Δ*acrF,* Δ*emrB*, Δ*emrY*, Δ*entS,* Δ*mdtF*, Δ*mdtBC*, Δ*macB*	70043438	07APR2021
NR-51908	*Haemophilus influenza*, NB65044-CDS0001	*acrB*::Km^R	70043424	23APR2021
NR-51947	*Klebsiella pneumoniae*, NB29002-JWK0080	Δ*tolC*	70043422	23APR2021
NR-51948	*Klebsiella pneumoniae*, NB29002-JWK0079	Δ*acrB*	70048194	20OCT2021

NR-51923 was produced by inoculation of 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 70043438.

NR-51908 was produced by inoculation of the deposited material into Haemophilus Test Medium broth with 25 µg per milliliter kanamycin and grown for 1 day at 37°C in an aerobic atmosphere with 5% CO_2. Broth inoculum was added to GC agar with 25 µg per milliliter kanamycin kolles, which were grown for 1 day at 37°C in an aerobic atmosphere with 5% CO_2 to produce lot 70043424.

NR-51947 was produced by inoculation of the deposited material into Tryptic Soy broth with 25 µg per milliliter kanamycin and grown for 1 day at 37°C in an aerobic atmosphere. Broth inoculum was added to Tryptic Soy agar with 25 µg per milliliter kanamycin kolles, which were grown for 1 day at 37°C in an aerobic atmosphere to produce this lot.

NR-51948 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 70048194.

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

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Table 2: *Escherichia coli*, Strain NB27079-CDY0099 (NR-51923)

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology	Gram-negative rods	Gram-negative rods
Colony morphology	Report results	Circular, convex, entire, smooth and cream (Figure 1a)
Motility (wet mount)	Report results	Motile
VITEK® MS (MALDI-TOF)	*E. coli*	*E. coli* (99.9%)
Antibiotic Susceptibility Profile		
Tryptic Soy broth with 25 µg/mL kanamycin	No growth	No growth
BD BBL™ Sensi-Disc™ susceptibility test disc 1 day at 35°C in an aerobic atmosphere on Mueller Hinton agar		
Gatifloxacin	Report results	31 to 35 mm
Novobiocin	Report results	25 mm
Etest® antibiotic test strips 1 day at 35°C in an aerobic atmosphere on Mueller Hinton agar		
Erythromycin	Report results	1.5-2 µg/mL
Kanamycin	Report results	1.5 µg/mL
Linezolid	Report results	4-6 µg/mL
Rifampin	Report results	3 µg/mL
Tetracycline	Report results	0.75 µg/mL
Trimethoprim	Report results	0.05 µg/mL
Sensititre™ GNX2F AST		
Colistin	Report results	≤ 0.025 µg/mL
Genotypic Analysis		
Digital DNA-DNA hybridization (dDDH)[1]	≥ 70% for species identification	*Escherichia coli* (75.4%)
Deletion of *acrB*, *acrD*, *acrF*, *emrB*, *emrY*, *entS*, *macB*, *mdtBC* and *mdtF*	Deletions 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]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. *E. coli,* DSM 30083 (GeneBank: KK583188.1) was used for dDDH analysis.

Table 3: *Haemophilus influenzae*, Strain NB65044-CDS0001 (NR-51908)

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology	Gram-negative rods	Gram-negative rods
Colony morphology	Report results	Circular, flat, entire, smooth and gray (Figure 1b)
Motility (wet mount)	Report results	Non-motile
VITEK® MS (MALDI-TOF)	*H. influenzae*	*H. influenzae* (99.9%)
Antibiotic Susceptibility Profile		
GC agar with 25 µg/mL kanamycin	Growth	Growth
Cefinase™ Paper Disc	Report results	Positive
Etest® antibiotic test strips 1 day at 35°C in an aerobic atmosphere with 5% CO_2 on Haemophilus Test Medium agar		
Clindamycin	Report results	0.5 µg/mL
Erythromycin	Report results	0.25 µg/mL

TEST	SPECIFICATIONS	RESULTS
Tetracycline	Report results	0.5 µg/mL
Genotypic Analysis		
Digital DNA-DNA hybridization (dDDH)[1]	≥ 70% for species identification	*Haemophilus influenza* (77%)
Insertional inactivation of *acrB*	Insertion cassette present	Pending
Purity 7 days at 37°C in an aerobic atmosphere with 5% CO_2 on Tryptic Soy agar with 5% sheep blood	Growth consistent with expected colony morphology	Growth consistent with expected colony morphology
Viability	Growth	Growth

[1]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. *H. influenzae,* strain NCTC8143 (GenBank: LN831035.1) was used for dDDH analysis.

Table 4: *Klebsiella pneumoniae*, Strain NB29002-JWK0080 (NR-51947)

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology	Gram-negative rods	Gram-negative rods
Colony morphology	Report results	Circular, slight peaked, entire, smooth, mucoid and cream (Figure 1c)
Motility BBL™ Motility Test Medium w/TTC Indicator for 1 day at 37°C in an aerobic atmosphere	Report results	Motile
VITEK® MS (MALDI-TOF)	*K. pneumoniae*	*K. pneumoniae* (99.9%)
Antibiotic Susceptibility Profile		
Tryptic Soy agar with 25 µg/mL kanamycin	Growth	Growth
BD BBL™ Sensi-Disc™ susceptibility test disc 1 day at 35°C in an aerobic atmosphere on Mueller Hinton agar		
Gatifloxacin	Report results	33 to 34 mm
Genotypic Analysis		
Digital DNA-DNA hybridization (dDDH)[1]	≥ 70% for species identification	*K. pneumoniae* (93.9%)
Deletion of *tolC*	*tolC* deletion 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]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. *K. pneumoniae,* strain, NCTC 9633 (GenBank: UAWR01000000) was used for dDDH alnalysis.

Table 5: *Klebsiella pneumoniae*, Strain NB29002-JWK0079 (NR-51948)

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology	Gram-negative rods	Gram-negative rods
Colony morphology	Report results	Circular, convex, entire, smooth, mucoid and cream (Figure 1d)
Motility BBL™ Motility Test Medium w/TTC Indicator for 1 day at 37°C in an aerobic atmosphere	Report results	Motile
VITEK® 2 Compact (GN card)	*K. pneumoniae* (≥ 89.9%)	*K. pneumoniae* (93%)
Antibiotic Susceptibility Profile		
BD BBL™ Sensi-Disc™ susceptibility test disc 1 day at 35°C in an aerobic atmosphere on Mueller Hinton agar		

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TEST	SPECIFICATIONS	RESULTS
Gatifloxacin Etest® antibiotic test strips 1 day at 35°C in an aerobic atmosphere on Mueller Hinton agar	Report results	30 mm
Kanamycin	Report results	≥ 256 µg/mL
Genotypic Analysis		
Digital DNA-DNA hybridization (dDDH)[1]	≥ 70% for species identification	*K. pneumoniae* (93.6%)
Deletion of *acrB*	*acrB* deletion	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]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. *K. pneumoniae* strain NCTC 9633 (GenBank: UAWR01000000) was used for dDDH alnalysis.

Figure 1a: NR-51923 Colony Morphology

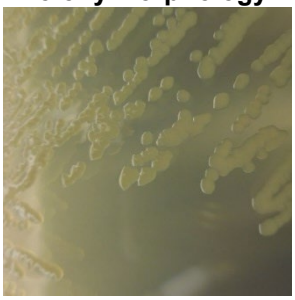


Figure 1b: NR-51908 Colony Morphology

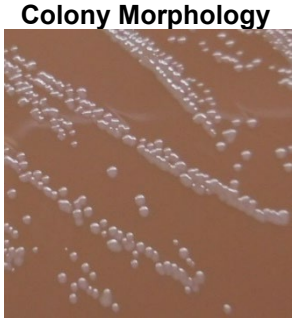


Figure 1c: NR-51947 Colony Morphology

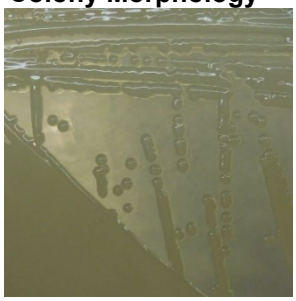
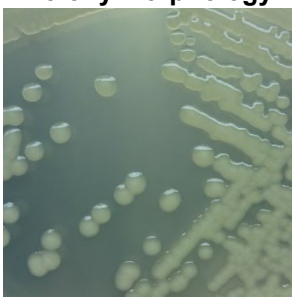


Figure 1d: NR-51948 Colony Morphology



/Sonia Bjorum Brower/
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