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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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COMPONENT NUMBER	DESCRIPTION	GENOTYPE	LOT NUMBER	DATE OF MANUFACTURE
NR-51923	Escherichia coli, NB27079-CDY0099	ΔacrB, ΔacrD, ΔacrF, ΔemrB, ΔemrY, ΔentS, ΔmdtF, ΔmdtBC, ΔmacB	70043438	07APR2021
NR-51908	Haemophilus influenza, NB65044-CDS0001	<i>acrB</i> ::Km ^R	70043424	23APR2021
NR-51947	Klebsiella pneumoniae, NB29002-JWK0080	∆tolC	70043422	23APR2021
NR-51948	Klebsiella pneumoniae, NB29002-JWK0079	∆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 μ g per milliliter kanamycin and grown for 1 day at 37°C in an aerobic atmosphere with 5% CO₂. Broth inoculum was added to GC agar with 25 μ g per milliliter kanamycin kolles, which were grown for 1 day at 37°C in an aerobic atmosphere with 5% CO₂ 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			
Frythromycin	Report results	1.5 to 2 µg/ml	
Kanamycin	Report results	1.5 ug/ml	
	Report results	A to 6 ug/ml	
Rifampin	Report results		
Tetracycline	Report results	$0.75 \mu g/mL$	
Trimethonrim	Report results	$0.05 \mu\text{g/mL}$	
Sonsititro™ GNY2E AST	Report results		
Colistin	Report results	≤ 0.025 µa/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	Growth consistent with expected colony	Growth consistent with expected	
7 days at 37°C in an aerobic atmosphere with and without 5% CO ₂ on Tryptic Soy agar	morphology	colony morphology	
Viability	Growth	Growth	

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." <u>Stand. Genomic Sci.</u> 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	

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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	Growth consistent with expected	Growth consistent with expected	
7 days at 37°C in an aerobic atmosphere with 5%	colony morphology	colony morphology	
CO ₂ on Tryptic Soy agar with 5% sheep blood			
Viability	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. *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	Report results	Motile
BBL™ Motility Test Medium w/TTC Indicator		
for 1 day at 37°C in an aerobic atmosphere		
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 <i>tolC</i>	to/C deletion present	Pending
Purity	Growth consistent with expected	Growth consistent with expected
7 days at 37°C in an aerobic atmosphere with and	colony morphology	colony morphology
without 5% CO ₂ on Tryptic Soy agar		
Viability	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. *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	Report results	Motile
BBL [™] Motility Test Medium w/TTC Indicator		
for 1 day at 37°C in an aerobic atmosphere		
VITEK [®] 2 Compact (GN card)	<i>K. pneumoniae</i> (≥ 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	Report results	30 mm
Etest [®] antibiotic test strips		
1 day at 35°C in an aerobic atmosphere on		
Mueller Hinton agar		
Kanamycin	Report results	≥ 256 µg/mL
Genotypic Analysis		
Digital DNA-DNA hybridization (dDDH) ¹	≥ 70% for species identification	K. pneumoniae (93.6%)
Deletion of <i>acrB</i>	<i>acrB</i> deletion	Pending
Purity	Growth consistent with expected	Growth consistent with expected
7 days at 37°C in an aerobic atmosphere with and	colony morphology	colony morphology
without 5% CO ₂ on Tryptic Soy agar		
Viability	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. *K. pneumoniae* strain NCTC 9633 (GenBank: UAWR01000000) was used for dDDH alnalysis.



Figure 1b: NR-51908 Colony Morphology



Figure 1c: NR-51947 Colony Morphology Figure 1d: NR-51948 Colony Morphology



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