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

Catalog No. NR-55641

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Manufacturer:
BEI Resources

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. pneumonia)*. These mutants were used in a mass spectroscopy-based cellular assay to optimize inhibitors of CoaD, a key bacterial enzyme involved in the coenzyme A (CoA-SH) biosynthesis pathway.[1,2,3] The panel contains the strains listed in Table 1.

Table 1: Mutant Strains

Item Number	Bacterial Strain	Genotype
NR-51923	*Escherichia coli* NB27079-CDY0099	Δ*acrB* Δ*acrD* Δ*acrF* Δ*emrB* Δ*emrY* Δ*entS* Δ*macB* Δ*mdtBC* Δ*mdtF*
NR-51908	*Haemophilus influenzae* NB65044-CDS0001	*acrB*::Km^R
NR-51947	*Klebsiella pneumoniae* NB29002-JWK0080	Δ*tolC*
NR-51948	*Klebsiella pneumoniae* NB29002-JWK0079	Δ*acrB*

NR-51923 was created by deletion of 9 genes encoding Resistance-Nodulation-Division (RND) family efflux pumps: *acrB*, *acrD*, *acrF*, *emrB*, *emrY*, *entS*, *macB*, *mdtBC* and *mdtF*, from the chromosome of *E. coli* strain BW25113. The genes were deleted sequentially via recombineering using DNA fragments containing a kanamycin marker *(aph)* flanked by homologous sequences of the targeted genes. The kanamycin marker was subsequently removed by FLP recombinase.[2]

NR-51908 was created by the insertional inactivation of *acrB*, in the chromosome of *H. influenzae strain* ATCC® 51907™.[3]

NR-51947 and NR-51948 were created by the deletion of *tolC* and *acrB*, respectively, from the chromosome of *K. pneumoniae* ATCC® 43816™ through recombineering using a DNA fragment containing a kanamycin marker *(aph)* flanked by homologous sequence upstream and downstream of *tolC* and *acrB*, respectively.[1]

Detailed information for each mutant strain, including antibiotic susceptibility profile, is available on the Certificate of Analysis.

Material Provided:
Each panel contains one vial of each of the bacterial strains in the panel. Each vial of *E. coli* and *K. pneumoniae* contains approximately 0.5 mL of bacterial culture in Tryptic Soy broth containing 25 µg per milliliter kanamycin supplemented with 10% glycerol. Each vial of NR-51947 contains approximately 0.5 mL of bacterial culture in Haemophilus Test broth containing 25 µg per milliliter kanamycin supplemented with 10% glycerol. Each vial of NR-51948 contains approximately 0.5 mL of bacterial culture in Haemophilus Test broth supplemented with 10% glycerol.

Note: If homogeneity is required for your intended use, please purify prior to initiating work.

Packaging/Storage:
Each isolate was packaged aseptically in cryovials. The product is provided frozen and should be stored at -60°C or colder immediately upon arrival. For long-term storage, the vapor phase of a liquid nitrogen freezer is recommended. Freeze thaw cycles should be avoided.

Growth Conditions:
E. coli and *K. pneumoniae*
Media:
Tryptic Soy broth containing 25 µg per milliliter kanamycin or equivalent
Tryptic Soy agar containing 25 µg per milliliter kanamycin or equivalent
Incubation:
Temperature: 37°C
Atmosphere: Aerobic
Propagation:
1. Keep vial frozen until ready for use, then thaw.
2. Transfer the entire thawed aliquot into a single tube of broth.
3. Use several drops of the suspension to inoculate an agar slant and/or plate.
4. Incubate the tube, slant and/or plate at 37°C for 1 day.

H. influenzae
Media:
Haemophilus Test broth containing 25 µg per milliliter kanamycin or equivalent
Haemophilus Test agar containing 25 µg per milliliter kanamycin or equivalent
Incubation:
Temperature: 37°C with 5% CO_2
Atmosphere: Aerobic
Propagation:
1. Keep vial frozen until ready for use, then thaw.
2. Transfer the entire thawed aliquot into a single tube of broth.
3. Use several drops of the suspension to inoculate an agar slant and/or plate.

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4. Incubate the tube, slant and/or plate at 37°C for 1 to 2 days.

Citation:

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: Antimicrobial Resistance Panel 2: Multiple Species Coenzyme A (CoA-SH) Biosynthesis Pathway, NR-55641."

Biosafety Level: 2

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories. 6th ed. Washington, DC: U.S. Government Printing Office, 2020; see www.cdc.gov/biosafety/publications/bmbl5/index.htm.

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References:

1. Rath, C. M., et al. "Optimization of CoaD Inhibitors against Gram-Negative Organisms through Targeted Metabolomics." ACS Infect. Dis. 4 (2018): 391-402. PubMed: 29243909.
2. Jones, A. K., et al. "Determinants of Antibacterial Spectrum and Resistance Potential of the Elongation Factor G Inhibitor Argyrin B in Key Gram-Negative Pathogens." Antimicrob. Agents and Chemother. 61 (2017): e02400-16. PubMed: 28096160.
3. Dean, C. R., et al. "Role of the AcrAB-TolC Efflux Pump in Determining the Susceptibility of *Haemophilus influenzae* to the Novel Peptide Deformylase Inhibitor LBM415." Antimicrob. Agents and Chemother. 49 (2005): 3129-35. PubMed: 16048914

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