

Certificate of Analysis for NR-55649

Antimicrobial Resistance Panel 10: Escherichia coli mrdA Mutants

Catalog No. NR-55649

Product Description:

NR-55649 consists of a 2-member panel of *Escherichia coli* (*E. coli*), strain BW25113 derivatives harboring clinically relevant mutations in *mrdA*, which encodes for penicillin-binding protein 2 (PBP2). These mutant strains were produced through recombination with PCR-amplified mutant *mrdA*. *E. coli*, strain NB27079-CDK0001 was created by the introduction of PCR amplified *mrdA* genomic sequence from drug-resistant *E. coli* clinical isolate ATCC® BAA-2471™. *E. coli*, strain NB27079-CDK0004 was created by the introduction of PCR amplified *mrdA* genomic sequence from *E. coli* clinical isolate IHMA.

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Table 1: Kit Components

| COMPONENT NUMBER | DESCRIPTION | POINT MUTATION | LOT NUMBER | MANUFACTURING DATE |
|------------------|---|----------------|------------|--------------------|
| NR-51873 | E. coli, strain NB27079- CDK0001 | PBP2 L573Q | 70047257 | 15SEP2021 |
| NR-51874 | <i>E. coli</i> , strain NB27079- CDK0004 | PBP2 V5221 | 70047259 | 15SEP2021 |

NR-51873 and NR-51874 were 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 lots 70047257 and 70047259, respectively. Quality control testing was completed under propagation conditions unless otherwise noted.

Table 2: E. coli, strain NB27079-CDK0001 (NR-51873)

| 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 Remel™ Motility Test Medium w/TTC | Report results | Motile |
| Indicator for 1 day at 37°C in an aerobic atmosphere | | |
| VITEK® MS (MALDI-TOF) | E. coli | E. coli (99.9%) |
| Antibiotic Susceptibility Profile ¹ | | |
| Etest® antibiotic test strips | | |
| 1 day at 35°C in an aerobic atmosphere on | | |
| Mueller Hinton agar | | |
| Aztreonam | Report results | Sensitive (0.094 µg/mL) |
| Ceftazidime | Report results | Sensitive (0.19 µg/mL) |
| Imipenem | Report results | Sensitive (1.0 µg/mL) |
| Meropenem | Report results | Sensitive (0.064 µg/mL) |
| Genotypic Analysis | | |
| Digital DNA-DNA hybridization (dDDH) ² | ≥ 70% for species identification | Escherichia coli (75.1%) |
| Detection of mrdA mutation | Mutation detected | 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 |

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Table 3: E. coli, strain NB27079-CDK0004 (NR-51874)

| TEST | SPECIFICATIONS | RESULTS |
|--|----------------------------------|--|
| Phenotypic Analysis | | |
| Cellular morphology | Gram-negative rods | Gram-negative rods |
| Colony morphology | Report results | Circular, convex, entire, smooth and cream (Figure 1b) |
| Motility (wet mount) | Report results | Motile |
| VITEK® MS (MALDI-TOF) | E. coli | E. coli (99.9%) |
| Antibiotic Susceptibility Profile ¹ | | |
| Etest® antibiotic test strips | | |
| 1 day at 35°C in an aerobic atmosphere on | | |
| Mueller Hinton agar | | |
| Aztreonam | Report results | Sensitive (0.064 – 0.094 μg/mL) |
| Ceftazidime | Report results | Sensitive (0.125 – 0.19 µg/mL) |
| Imipenem | Report results | Sensitive (0.75 µg/mL) |
| Meropenem | Report results | Sensitive (0.032 µg/mL) |
| Genotypic Analysis | | |
| Digital DNA-DNA hybridization (dDDH) ² | ≥ 70% for species identification | Escherichia coli (75.1%) |
| Detection of mrdA mutation | Mutation detected | Pending |
| Purity | Growth consistent with expected | Growth consistent with expected |
| 7 days at 37°C in an aerobic atmosphere with | colony morphology | colony morphology |
| and without 5% CO ₂ on Tryptic Soy agar | | |
| Viability | Growth | Growth |

¹Minimum Inhibitory Concentration (MIC); MIC Interpretation Guideline: CLSI M100-S28 (2018)

Figure 1a: NR-51873 Colony Morphology

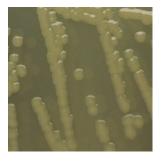


Figure 1b: NR-51874 Colony Morphology



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18 JUL 2022

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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.