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# Certificate of Analysis for NR-9546

## Diarrheagenic *Escherichia coli* Nucleic Acid Panel

## Catalog No. NR-9546

**Product Description:** NR-9546 consists of nucleic acid from five organisms representing different diarrheagenic *Escherichia coli* (*E. coli*) pathotypes. The indicated pathotypes have been confirmed by PCR amplification of marker sequences.

## Lot: 58047884

**NR-2647, Lot 5107284 (ETEC; Manufactured 22FEB2006)[1]**

| TEST | SPECIFICATIONS | RESULTS |
|---|---|---|
| **Genotypic Analysis** | | |
| Sequencing of 16S rRNA gene (~ 500 bp) | Identical to NR-4<br>Consistent with *Escherichia coli* | Identical to NR-4<br>Consistent with *Escherichia coli*[2] |
| PCR amplification of plasmid markers[3] | | |
| *elt* (pJY11) | Report results | Positive |
| *esth* (pCS1) | Report results | Positive |
| *estp* (pCS1) | Report results | Positive |
| EAF (pEAF) | Negative | Negative |
| *bfpA* (pEAF) | Negative | Negative |
| *invE* (pINV) | Negative | Negative |
| CVD432 (pAA) | Negative | Negative |
| *aggR* (pAA) | Negative | Negative |
| PCR amplification of chromosomal markers[3] | | |
| *eaeA* | Negative | Negative |
| *stx1* | Negative | Negative |
| *stx2* | Negative | Negative |
| *astA* | Report results | Positive |
| **Agarose Gel Electrophoresis** | High molecular weight chromosomal DNA | High molecular weight chromosomal DNA (Figure 1A) |
| **Concentration by PicoGreen® Measurement** | 4 to 6 µg in 25 to 100 µL per vial | 4.8 µg in 62 µL per vial (77 µg/mL) |
| **Functional Activity by PCR Amplification** | | |
| 16S ribosomal RNA gene | ~ 1500 bp amplicon | ~ 1500 bp amplicon |
| Virulence markers[3] | | |
| *elt* (pJY11) | ~ 263 bp amplicon | ~ 263 bp amplicon |
| *esth* (pCS1) | ~ 179 bp amplicon | ~ 179 bp amplicon |
| *estp* (pCS1) | ~ 123 bp amplicon | ~ 123 bp amplicon |
| *astA* (chromosome) | ~ 106 bp amplicon | ~ 106 bp amplicon |
| **$OD_{260}/OD_{280}$ Ratio** | 1.7 to 1.9 | 1.8 |
| **Bacterial Inactivation** | | |
| 10% of total yield plated on Nutrient Agar and incubated for 7 days at 37°C | No viable bacteria detected | No viable bacteria detected |

[1]The bacterial preparation used for extraction of genomic DNA was produced by inoculation of BEI Resources NR-4 (Lot 3561329) into LB Broth (ATCC® 60-2100). After incubation for 16 hours at 37°C, genomic DNA was extracted using proprietary technology.
[2]Also consistent with *Shigella* species.
[3]Kimata, K., et al. "Rapid Categorization of Pathogenic *Escherichia coli* by Multiplex PCR." Microbiol. Immunol. 49 (2005): 485–492. PubMed: 15965295.

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[4]An extraction procedure was used that has been shown to consistently inactivate 100% of Gram-negative bacteria.

**NR-2648, Lot 5107305 (EHEC; Manufactured 07FEB2006)[1]**

| TEST | SPECIFICATIONS | RESULTS |
|---|---|---|
| **Genotypic Analysis** | | |
| Sequencing of 16S rRNA gene (~ 500 bp) | Identical to NR-11<br>Consistent with *Escherichia coli* | Identical to NR-11<br>Consistent with *Escherichia coli*[2] |
| PCR amplification of plasmid markers | | |
| *hylA* (pO157) | Positive | Positive |
| *elt* (pJY11) | Negative | Negative |
| *esth* (pCS1) | Negative | Negative |
| *estp* (pCS1) | Negative | Negative |
| EAF (pEAF) | Negative | Negative |
| *bfpA* (pEAF) | Negative | Negative |
| *invE* (pINV) | Negative | Negative |
| CVD432 (pAA) | Negative | Negative |
| *aggR* (pAA) | Negative | Negative |
| PCR amplification of chromosomal markers | | |
| *eaeA* | Positive | Positive |
| *stx1* | Report results | Positive |
| *stx2* | Report results | Positive |
| *astA* | Report results | Negative |
| **Agarose Gel Electrophoresis** | High molecular weight chromosomal DNA | High molecular weight chromosomal DNA (Figure 1B) |
| **Concentration by PicoGreen® Measurement** | 4 to 6 µg in 25 to 100 µL per vial | 5.1 µg in 77 µL per vial (66 µg/mL) |
| **Functional Activity by PCR Amplification** | | |
| 16S ribosomal RNA gene | ~ 1500 bp amplicon | ~ 1500 bp amplicon |
| Virulence markers[3] | | |
| *hylA* (pO157) | ~ 3200 bp amplicon | ~ 3200 bp amplicon |
| *eaeA* (chromosome) | ~ 526 bp amplicon | ~ 526 bp amplicon |
| *stx1* (chromosome) | ~ 349 bp amplicon | ~ 349 bp amplicon |
| *sx2* (chromosome) | ~ 404 bp amplicon | ~ 404 bp amplicon |
| **$OD_{260}/OD_{280}$ Ratio** | 1.7 to 1.9 | 1.9 |
| **Bacterial Inactivation** | | |
| 10% of total yield plated on Nutrient Agar and incubated for 7 days at 37°C | No viable bacteria detected | No viable bacteria detected |

[1]The bacterial preparation used for extraction of genomic DNA was produced by inoculation of BEI Resources NR-11 (Lot 3560110) into LB Broth (ATCC® 60-2100). After incubation for 16 hours at 37°C, genomic DNA was extracted using proprietary technology.
[2]Also consistent with *Shigella* species.
[3]Kimata, K., et al. "Rapid Categorization of Pathogenic *Escherichia coli* by Multiplex PCR." Microbiol. Immunol. 49 (2005): 485–492. PubMed: 15965295.
[4]An extraction procedure was used that has been shown to consistently inactivate 100% of Gram-negative bacteria.

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# Certificate of Analysis for NR-9546

**NR-3050, Lot 7642439 (Atypical EPEC; Manufactured 06OCT2006)[1]**

| TEST | SPECIFICATIONS | RESULTS |
|---|---|---|
| **Genotypic Analysis** | | |
| Sequencing of 16S rRNA gene (~ 500 bp) | Consistent with *Escherichia coli* | Consistent with *Escherichia coli*[2] |
| PCR amplification of plasmid markers | | |
| EAF (pEAF) | Report results | Positive |
| *bfpA* (pEAF) | Report results | Positive |
| *elt* (pJY11) | Negative | Negative |
| *esth* (pCS1) | Negative | Negative |
| *estp* (pCS1) | Negative | Negative |
| *invE* (pINV) | Negative | Negative |
| CVD432 (pAA) | Negative | Negative |
| *aggR* (pAA) | Negative | Negative |
| PCR amplification of chromosomal markers | | |
| *eaeA* | Report results | Negative |
| *stx1* | Negative | Negative |
| *stx2* | Negative | Negative |
| *astA* | Report results | Negative |
| **Agarose Gel Electrophoresis** | High molecular weight chromosomal DNA | High molecular weight chromosomal DNA (Figure 1C) |
| **Concentration by PicoGreen® Measurement** | 4 to 6 μg in 25 to 100 μL per vial | 5.4 μg in 35 μL per vial (155 μg/mL) |
| **Functional Activity by PCR Amplification** | | |
| 16S ribosomal RNA gene | ~ 1500 bp amplicon | ~ 1500 bp amplicon |
| Virulence markers[3] | | |
| EAF (pEAF) | ~ 153 bp amplicon | ~ 153 bp amplicon |
| *bfpA* (pEAF) | ~ 209 bp amplicon | ~ 209 bp amplicon |
| **$OD_{260}/OD_{280}$ Ratio** | 1.7 to 1.9 | 1.9 |
| **Bacterial Inactivation** | | |
| 10% of total yield plated on Trypticase Soy Agar and incubated for 7 days at 37°C | No viable bacteria detected | No viable bacteria detected |

[1]The bacterial preparation used for extraction of genomic DNA was produced by inoculation of BEI Resources NR-99 (Lot 3663825) into LB Broth (ATCC® 60-2100). After incubation for 19 hours at 37°C, genomic DNA was extracted using proprietary technology.
[2]Also consistent with *Shigella* species.
[3]Kimata, K., et al. "Rapid Categorization of Pathogenic *Escherichia coli* by Multiplex PCR." Microbiol. Immunol. 49 (2005): 485–492. PubMed: 15965295.
[4]An extraction procedure was used that has been shown to consistently inactivate 100% of Gram-negative bacteria.

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# Certificate of Analysis for NR-9546

**NR-3051, Lot 7642440 (EIEC; Manufactured 06OCT2006)[1]**

| TEST | SPECIFICATIONS | RESULTS |
|---|---|---|
| **Genotypic Analysis** | | |
| Sequencing of 16S rRNA gene (~ 1350 bp) | Consistent with *Escherichia coli* | Consistent with *Escherichia coli*[2] |
| PCR amplification of plasmid markers | | |
| *invE* (pINV) | Positive | Positive |
| *elt* (pJY11) | Negative | Negative |
| *esth* (pCS1) | Negative | Negative |
| *estp* (pCS1) | Negative | Negative |
| EAF (pEAF) | Negative | Negative |
| *bfpA* (pEAF) | Negative | Negative |
| CVD432 (pAA) | Negative | Negative |
| *aggR* (pAA) | Negative | Negative |
| PCR amplification of chromosomal markers | | |
| *eaeA* | Negative | Negative |
| *stx1* | Negative | Negative |
| *stx2* | Negative | Negative |
| *astA* | Report results | Negative |
| **Agarose Gel Electrophoresis** | High molecular weight chromosomal DNA | High molecular weight chromosomal DNA (Figure 1D) |
| **Concentration by PicoGreen® Measurement** | 4 to 6 µg in 25 to 100 µL per vial | 5.2 µg in 49 µL per vial (105 µg/mL) |
| **Functional Activity by PCR Amplification** | | |
| 16S ribosomal RNA gene | ~ 1500 bp amplicon | ~ 1500 bp amplicon |
| Virulence markers[3] | | |
| *invE* (pINV) | ~ 293 bp amplicon | ~ 293 bp amplicon |
| **$OD_{260}/OD_{280}$ Ratio** | 1.7 to 1.9 | 1.8 |
| **Bacterial Inactivation** | | |
| 10% of total yield plated on Trypticase Soy Agar and incubated for 7 days at 37°C | No viable bacteria detected | No viable bacteria detected |

[1]The bacterial preparation used for extraction of genomic DNA was produced by inoculation of BEI Resources NR-100 (Lot 3670406) into LB Broth (ATCC® 60-2100). After incubation for 19 hours at 37°C, genomic DNA was extracted using proprietary technology.
[2]Also consistent with *Shigella* species.
[3]Kimata, K., et al. "Rapid Categorization of Pathogenic *Escherichia coli* by Multiplex PCR." Microbiol. Immunol. 49 (2005): 485–492. PubMed: 15965295.
[4]An extraction procedure was used that has been shown to consistently inactivate 100% of Gram-negative bacteria.

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# Certificate of Analysis for NR-9546

**NR-3052, Lot 7642443 (EAEC; Manufactured 06OCT2006)[1]**

| TEST | SPECIFICATIONS | RESULTS |
|---|---|---|
| **Genotypic Analysis** | | |
| Sequencing of 16S rRNA gene (~ 500 bp) | Consistent with *Escherichia coli* | Consistent with *Escherichia coli*[2] |
| PCR amplification of plasmid markers | | |
| CVD432 (pAA) | Report results | Positive |
| *aggR* (pAA) | Report results | Positive |
| *elt* (pJY11) | Report results | Negative |
| *esth* (pCS1) | Report results | Negative |
| *estp* (pCS1) | Report results | Negative |
| EAF (pEAF) | Negative | Negative |
| *bfpA* (pEAF) | Negative | Negative |
| *invE* (pINV) | Negative | Negative |
| PCR amplification of chromosomal markers | | |
| *eaeA* | Negative | Negative |
| *stx1* | Negative | Negative |
| *stx2* | Negative | Negative |
| *astA* | Report results | Negative |
| **Agarose Gel Electrophoresis** | High molecular weight chromosomal DNA | High molecular weight chromosomal DNA (Figure 1E) |
| **Concentration by PicoGreen® Measurement** | 4 to 6 µg in 25 to 100 µL per vial | 5.2 µg in 44 µL per vial (117 µg/mL) |
| **Functional Activity by PCR Amplification** | | |
| 16S ribosomal RNA gene | ~ 1500 bp amplicon | ~ 1500 bp amplicon |
| Virulence markers[3] | | |
| CVD432 (pAA) | ~ 690 bp amplicon | ~ 690 bp amplicon |
| *aggR* (pAA) | ~ 434 bp amplicon | ~ 434 bp amplicon |
| **$OD_{260}/OD_{280}$ Ratio** | 1.7 to 1.9 | 1.9 |
| **Bacterial Inactivation** | | |
| 10% of total yield plated on Trypticase Soy Agar and incubated for 7 days at 37°C | No viable bacteria detected | No viable bacteria detected |

[1]The bacterial preparation used for extraction of genomic DNA was produced by inoculation of BEI Resources NR-102 (Lot 3670409) into LB Broth (ATCC® 60-2100). After incubation for 19 hours at 37°C, genomic DNA was extracted using proprietary technology.
[2]Also consistent with *Shigella* species.
[3]Kimata, K., et al. "Rapid Categorization of Pathogenic *Escherichia coli* by Multiplex PCR." Microbiol. Immunol. 49 (2005): 485–492. PubMed: 15965295.
[4]An extraction procedure was used that has been shown to consistently inactivate 100% of Gram-negative bacteria.

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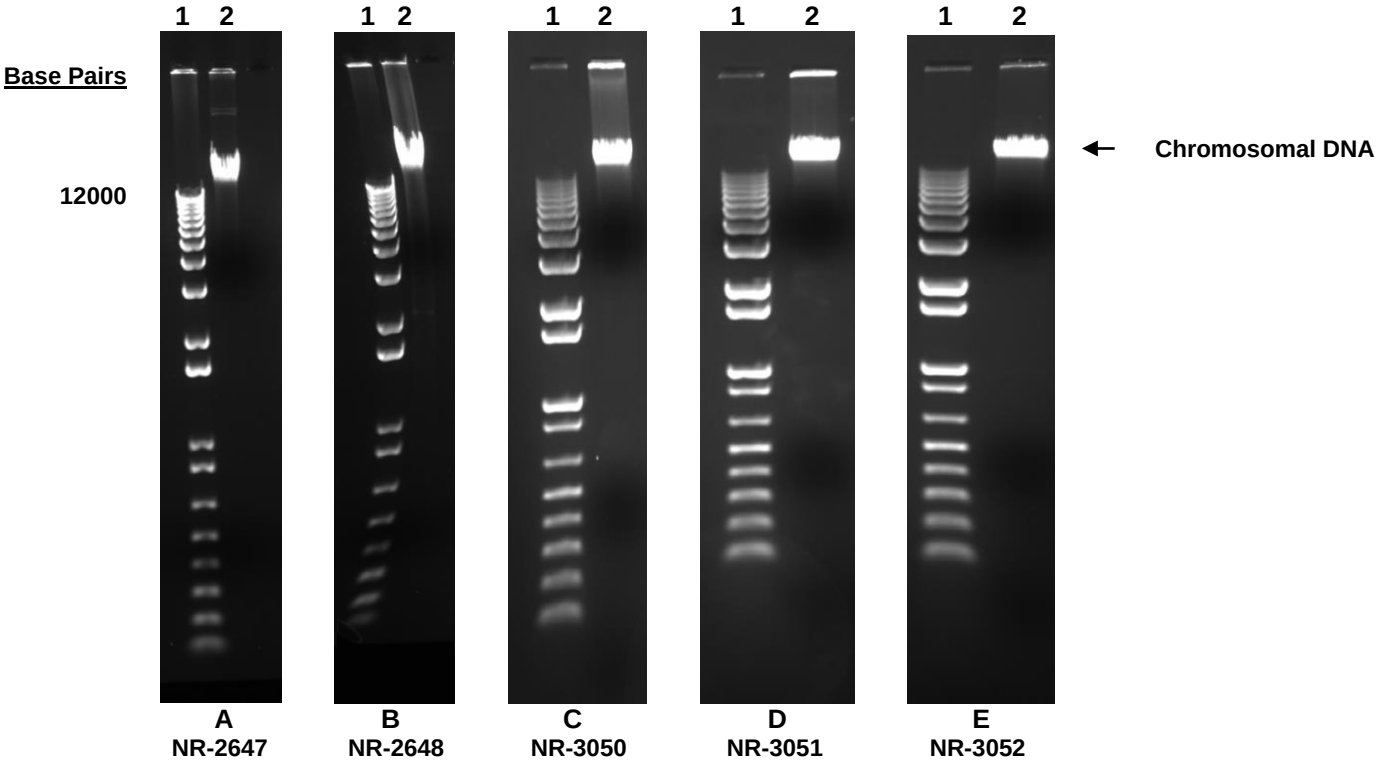
# Certificate of Analysis for NR-9546




**Lane 1: Invitrogen™ 1Kb DNA Ladder (A, B) or 1Kb Plus DNA Ladder (C, D, E)**
**Lane 2: 200 ng of indicated nucleic acid**

**Date:** 07 MAR 2008 **Signature:** Signature on File

**Title:** Technical Manager, BEI Authentication or designee

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