

Diarrheagenic *Escherichia coli* 12-Target Multiplex PCR Primers for Virulence Typing

Catalog No. NR-12207

Product Description: NR-12207 consists of 12 sets of PCR primers that specifically amplify a region of the virulence targets of 5 *Escherichia coli* (*E. coli*) pathotypes: enterohemorrhagic *E. coli* (EHEC); enteropathogenic *E. coli* (EPEC); enterotoxigenic *E. coli* (ETEC); enteroinvasive *E. coli* (EIEC) and enteroaggregative *E. coli* (EAggEC). Note: *E. coli* 12-Target Multiplex PCR 10X Buffer (BEI Resources NR-13440) will be provided with your shipment of NR-12207.

Lot: 58459952

Manufacturing Date: 12FEB2009

TEST	SPECIFICATIONS	RESULTS
PCR Amplification and Sequencing^{1,2} Amplicon size CVD 432 ² <i>eaeA</i> <i>aggR</i> ² <i>stx2</i> <i>stx1</i> <i>invE</i> <i>elt</i> <i>bfpA</i> ² <i>esth</i> ² EAF ² <i>estp</i> <i>astA</i> ² NCBI blast of sequence	Expected size ~ 632 bp ~ 526 bp ~ 438 bp ~ 403 bp ~ 348 bp ~ 292 bp ~ 264 bp ~ 197 bp ~ 180 bp ~ 152 bp ~ 121 bp ~ 106 bp Consistent with sequence	Expected size (Figure 1) ~ 632 bp ~ 526 bp ~ 438 bp ~ 403 bp ~ 348 bp ~ 292 bp ~ 264 bp ~ 197 bp ~ 180 bp ~ 152 bp ~ 121 bp ~ 106 bp Consistent with sequence
Specificity NR-3050 (EPEC) NR-3051 (EIEC) NR-3052 (EaggEC) NR-2648 (EHEC) NR-2647 (ETEC) NR-2654 (Uropathogenic <i>E. coli</i> , negative control) No template – molecular biology grade water	Specific for virulence target(s) <i>eaeA</i> , <i>bfpA</i> , EAF <i>invE</i> CVD432, <i>aggR</i> <i>eaeA</i> , <i>stx1</i> , <i>stx2</i> <i>elt</i> , <i>esth</i> , <i>estp</i> , <i>astA</i> No amplicons No amplicons	Specific for virulence target(s) (Figure 1) <i>eaeA</i> , <i>bfpA</i> , EAF <i>invE</i> CVD432, <i>aggR</i> <i>eaeA</i> , <i>stx1</i> , <i>stx2</i> <i>elt</i> , <i>esth</i> , <i>estp</i> , <i>astA</i> No amplicons No amplicons

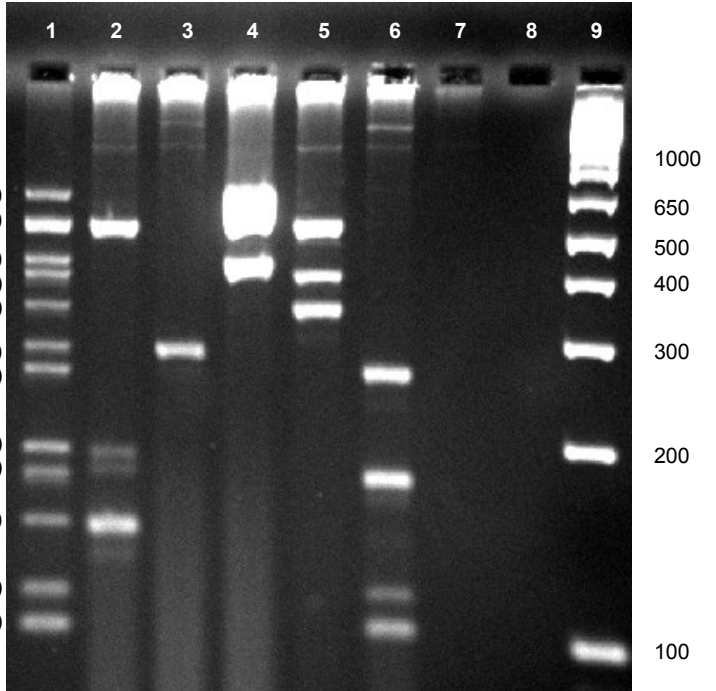
¹Genomic DNA from BEI Resources NR-2647 was used as a template for ETEC virulence targets, NR-2648 was used as template for EHEC virulence targets, NR-3050 was used as a template for EPEC virulence targets, NR-3051 was used as a template for EIEC virulence targets and NR-3052 was used as template for EAggEC virulence targets.

²The primers for CVD 432, *aggR*, *bfpA*, *esth*, EAF and *astA* are described in Kimata, K., et al. "Rapid Categorization of Pathogenic *Escherichia coli* by Multiplex PCR." *Microbiol. Immunol.* 49 (2005): 485-492. PubMed: 15965295. The primers for the remaining virulence markers were designed at BEI Resources.

Certificate of Analysis for NR-12207

Figure 1: Virulence Typing with 12-Target Multiplex PCR Primers and Diarrheagenic *E. coli* Templates

Lane 1: Diarrheagenic <i>E. coli</i> Virulence Target Ladder (BEI Resources NR-12150)	
Lane 2: NR-3050 (EPEC template)	CVD432 (632 bp)
Lane 3: NR-3051 (EIEC template)	<i>eaeA</i> (526 bp)
Lane 4: NR-3052 (EaggEC template)	<i>aggR</i> (438 bp)
Lane 5: NR-2648 (EHEC template)	<i>stx2</i> (403 bp)
Lane 6: NR-2647 (ETEC template)	<i>stx1</i> (348 bp)
Lane 7: NR-2654 (Uropathogenic <i>E. coli</i> , negative control template)	<i>invE</i> (292 bp)
Lane 8: No template – molecular biology grade water	<i>elt</i> (262 bp)
Lane 9: Invitrogen™ 1Kb Plus DNA Ladder™	<i>bfpA</i> (197 bp)
	<i>esth</i> (180 bp)
	EAF (152 bp)
	<i>estp</i> (121 bp)
	<i>astA</i> (106 bp)



Date: 24 APR 2012

Signature: *Dorothy C. Young*

Title: Technical Manager, BEI Authentication or designee

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