

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

Catalog No. NR-12207

For research use only. Not for human use.

Contributor and Manufacturer:

BEI Resources

Product Description:

Diarrheagenic *Escherichia coli* (*E. coli*) are classified into several pathogenic groups based on their virulence characteristics.¹ NR-12207 consists of 12 sets of PCR primers that specifically amplify a region of the virulence targets of 5 *E. coli* pathotypes: enterohemorrhagic *E. coli* (EHEC); enteropathogenic *E. coli* (EPEC); enterotoxigenic *E. coli* (ETEC); enteroinvasive *E. coli* (EIEC) and enteroaggregative *E. coli* (EAggEC). Each primer set is available individually by requesting the indicated BEI Resources NR number (Table 1). Additionally, a diarrheagenic *E. coli* DNA ladder is available (BEI Resources NR-12150) that can be used for identification of 12 *E. coli* virulence markers (sizes range from approximately 100 to 650 bps) and for determination of the diarrheagenic pathotype.

Table 1

Cat. No.	Virulence Target	Pathotype
NR-12195	CVD 432	EAggEC
NR-12196	<i>eaeA</i>	EHEC, EPEC
NR-12197	<i>aggR</i>	EAggEC
NR-12198	<i>stx2</i>	EHEC
NR-12199	<i>stx1</i>	EHEC
NR-12200	<i>invE</i>	EIEC
NR-12201	<i>elt</i>	ETEC
NR-12202	<i>bfpA</i>	EPEC
NR-12203	<i>esth</i>	ETEC
NR-12204	EAF	EPEC
NR-12205	<i>estp</i>	ETEC
NR-12206	<i>astA</i>	EHEC, EAggEC, ETEC, EPEC
NR-12150	Ladder	N/A

Note: *E. coli* pathotypes do not always contain all of the indicated virulence markers.¹

Material Provided:

Each vial contains approximately 80 to 100 µL of a mixture of forward and reverse primers in TE buffer (pH 7.0).

Note: *E. coli* 12-Target Multiplex PCR 10X Buffer (BEI Resources NR-13440) will be provided with your shipment of NR-12207.

Packaging/Storage:

NR-12207 was packaged aseptically in screw-capped plastic cryovials. The product is provided frozen on dry ice and should be stored at -60°C or colder immediately upon arrival. Freeze-thaw cycles should be minimized.

Citation:

Acknowledgment for publications should read “The following reagent was obtained through BEI Resources, NIAID, NIH: Diarrheagenic *Escherichia coli* 12-Target Multiplex PCR Primers for Virulence Typing, NR-12207.”

Biosafety Level: 1

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. 5th ed. Washington, DC: U.S. Government Printing Office, 2009; see www.cdc.gov/biosafety/publications/bmb15/index.htm.

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Microbiol. Immunol. 49 (2005): 485-492. PubMed: 15965295.

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References:

1. Kimata, K., et al. "Rapid Categorization of Pathogenic *Escherichia coli* by Multiplex PCR."



APPENDIX I

***E. coli* 12- Target Multiplex PCR Primers**

Recommended Reagents/Equipment

Reagent	Source	Catalog #
<i>E. coli</i> 12-Target Multiplex Primers	BEI Resources	NR -12207
Positive Control Template, Genomic DNA from <i>E. coli</i> , Strains H10407 (ETEC), EDL933 (EHEC), CDC (EPEC), 1885-77 (EIEC) and NCDC U14-41 (EAggEC)	BEI Resources	NR-2647, NR-2648, NR-3050, NR-3051 and NR-3052, respectively
10X PCR Buffer	BEI Resources	NR-13440
GoTaq® Polymerase	Promega	M500B
dNTP Mix	Promega	U151
Molecular Biology Grade Water	ATCC®	60-2645

Reaction Mix¹

Reagent	Stock Concentration	Volume per Reaction (µL)
Molecular Biology Grade Water	---	14
10X PCR Buffer	10X	3
dNTP Mix	10 mM each	0.6
GoTaq® Polymerase	---	0.8
Primers ²	10 µM (each primer)	1.6
Template DNA	10 ng per µL	10
		Total – 30 µL

¹Reaction mix should be kept on bench-top cooler until ready for use.

²Primers are supplied at working stock concentrations.

Cycling Protocol

Cycle	# of Repeats	Step	Conditions
1	1	1	94°C for 5 minutes
2	30	1	94°C for 1 minute
		2	55°C for 1 minute
		3	72°C for 1 minute
3	1	1	72°C for 7 minutes
4	Indefinite	1	Hold at 4°C