

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

Catalog No. NR-13439

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Contributor:

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Product Description:

Diarrheagenic *Escherichia coli* (*E. coli*) are classified into several pathogenic groups based on their virulence genes. NR-13439 is designed to detect 12 of these virulence genes (CVD432, *eaeA*, *aggR*, *stx2*, *stx1*, *invE*, *elt*, *bfpA*, *esth*, EAF, *estp* and *astA*)¹ and consists of the following components (Table 1): 1) multiplex PCR primers that specifically amplify a region of each of these 12 virulence genes, 2) genomic DNAs from five *E. coli* strains with identified virulence genes (enteropathogenic, EPEC; enteroinvasive, EIEC; enteroaggregative, EAEC; enterohemorrhagic, EHEC; enterotoxigenic, ETEC), 3) genomic DNA from an *E. coli* strain that contains none of the 12 virulence genes (uropathogenic, UPEC), 4) multiplex PCR 10X buffer and 5) a virulence target ladder that can be used for identification of the virulence genes using gel electrophoresis.

Table 1

Cat. No.	Description
NRC-2200	12-Target Multiplex PCR Primers
NR-3050	Genomic DNA from <i>E. coli</i> , Strain CDC (EPEC)
NR-3051	Genomic DNA from <i>E. coli</i> , Strain 1885-77 (EIEC)
NR-3052	Genomic DNA from <i>E. coli</i> , Strain NCDC U14-41 (EAEC)
NR-2648	Genomic DNA from <i>E. coli</i> , Strain EDL933 (EHEC)
NR-2647	Genomic DNA from <i>E. coli</i> , Strain H10407 (ETEC)
NR-2656	Genomic DNA from <i>E. coli</i> , Strain CFT073 (UPEC)
NR-13440	12-Target Multiplex PCR 10X Buffer
NRC-2187	Virulence Target Ladder

Material Provided:

Each kit contains 80 µL to 100 µL of a mixture of 12-target multiplex PCR primers in TE buffer (pH 7.0); 30 µL to 80 µL of genomic DNA in TE buffer (pH ~ 7.4), 50 µL of 12-target multiplex PCR 10X buffer and 50 µL of virulence target ladder in TE buffer (pH 7.0).

Packaging/Storage:

NR-13439 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 the NIH Biodefense and Emerging Infections Research Resources Repository, NIAID,

NIH: Diarrheagenic *Escherichia coli* 12-Target Multiplex PCR for Virulence Typing Assay, NR-13439.”

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, 2007; see www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm.

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References:

1. 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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APPENDIX I

***E. coli* 12- Target Multiplex PCR Virulence Typing Assay**

Recommended Reagents/Equipment

Reagent	Source	Catalog #
12-Target Multiplex PCR Primers	BEI Resources	NRC-2200 (NR -12207)
<u>Positive Control Templates</u> Genomic DNA from <i>E. coli</i> strains CDC (EPEC), 1885-77 (EIEC), NCDC U14-41 (EAEC), EDL933 (EHEC), H10407 (ETEC)	BEI Resources	NR-3050, NR-3051 and NR-3052, NR-2648, NR-2647, respectively
<u>Negative Control Template</u> Genomic DNA from <i>E. coli</i> , strain CFT073 (UPEC)	BEI Resources	NR-2656
12-Target Multiplex PCR 10X Buffer	BEI Resources	NR-13440
<i>iTaq</i> [®] Polymerase	Bio-Rad	170-8870
dNTP Mix	Bio-Rad	170-8874
Molecular Biology Grade Water	Multiple Vendors	---
Virulence Target Ladder	BEI Resources	NRC-2187 (NR-12150)

Reaction Mix¹

Reagent	Stock Concentration	Volume per Reaction (µL)
Molecular Biology Grade Water	---	14
12-Target Multiplex PCR 10X Buffer	10X	3
dNTP Mix	10 mM each	0.6
<i>iTaq</i> [®] Polymerase	---	0.8
12-Target Multiplex PCR Primers ²	10 µM (each primer)	1.6
Template DNA	10 ng per µL	10
		Total – 30 µL

¹All reaction mix material should be kept ice cold 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