

***Escherichia coli* Virulence Target *esth* Primers**

Catalog No. NR-12203

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Contributor:

NIH Biodefense and Emerging Infections Research Resources Repository, NIAID, NIH

Product Description:

Diarrheagenic *Escherichia coli* (*E. coli*) are classified into several pathogenic groups based on their virulence characteristics. NR-12203 contains forward and reverse primers that specifically amplify a region of the virulence target heat stable enterotoxin (isolated from humans), *esth*, found on plasmid pCS1 of enterotoxigenic *E. coli* (ETEC).¹

Material Provided:

Each vial contains approximately 80 µL of a mixture of forward and reverse primers in TE buffer (pH 7.0). The concentration is shown on the Certificate of Analysis.

Note: *E. coli* 12-Target Multiplex PCR 10X Buffer (BEI Resources NR-13440) will be provided with your shipment of NR-12203.

Packaging/Storage:

Primers were packaged aseptically in screw-capped plastic cryovials. The product is provided frozen on dry ice and should be stored at -60°C or colder 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: *Escherichia coli* Virulence Target *esth* Primers, NR-12203."

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/bmb15/bmb15toc.htm.

Disclaimers:

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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.
2. Yamamoto, T. and T. Yokota. "Plasmids of Enterotoxigenic *Escherichia coli* H10407: Evidence for Two Heat-Stable Enterotoxin Genes and a Conjugal Transfer System." J. Bacteriol. 153 (1983): 1352-1360. PubMed: 6298182.

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APPENDIX I

***E. coli* Virulence Target *esth* Primers**

Recommended Reagents/Equipment

Reagent	Source	Catalog #
<i>E. coli</i> Virulence Target <i>esth</i> Primers	BEI Resources	NR -12203
Positive Control Template, Genomic DNA from <i>E. coli</i> , Strain H10407	BEI Resources	NR-2647
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	---	19.2
10X PCR Buffer	10X	3
dNTP Mix	10 mM each	0.6
GoTaq® Polymerase	---	0.2
Primers ²	10 µM (each primer)	5
Template DNA	1 ng per µL	2
		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	52°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