

## ***Escherichia coli* Virulence Target *estp* Primers**

### **Catalog No. NR-12205**

This reagent is the tangible property of the U.S. Government.

### **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. NR-12205 contains forward and reverse primers that specifically amplify a region of the virulence target heat stable enterotoxin (isolated from pigs), *estp*, found on plasmid pCS1 of enterotoxigenic *E. coli* (ETEC).

#### **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-12205.

#### **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 BEI Resources, NIAID, NIH: *Escherichia coli* Virulence Target *estp* Primers, NR-12205."

#### **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/bmbl5/index.htm](http://www.cdc.gov/biosafety/publications/bmbl5/index.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.
3. Sekizaki, T., H. Akashi and N. Terakado. "Nucleotide Sequences of the Genes for *Escherichia coli* Heat-Stable Enterotoxin I of Bovine, Avian, and Porcine Origins." [Am. J. Vet. Res.](#) 46 (1985): 909-912. PubMed: 2990268.

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APPENDIX I

*E. coli* Virulence Target *estp* Primers

Recommended Reagents/Equipment

Reagent	Source	Catalog #
<i>E. coli</i> Virulence Target <i>estp</i> Primers	BEI Resources	NR -12205
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 Mix<sup>1</sup>

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 <sup>2</sup>	10 µM (each primer)	5
Template DNA	1 ng per µL	2
		Total – 30 µL

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

<sup>2</sup>Primers 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