

## ***Escherichia coli* (E.coli) O157:H7 Quantified Bacterial DNA Standards (STX1 and STX2 genes)**

**Product # 28302**

*E. coli* O157:H7 is a rod-shaped, gram-negative bacterium. It is an enterohemorrhagic strain of the common *E. coli* bacterium and infection by the O157:H7 strain is commonly associated with hemorrhagic colitis. *E. coli* O157:H7 is recognized by its somatic (cell wall) antigen (O157) and its flagella antigen (H7). In addition, *E. coli* O157:H7 is known to produce Shiga-like toxins, which cause severe symptoms. While most patients can recover from the infection, up to 15% of the patients may develop hemolytic uremic syndrome, a type of kidney failure that could be fatal. Infection of *E. coli* O157:H7 usually results from consumption of poorly prepared food including undercooked meat (particularly ground beef), untreated water or raw unpasteurized milk.

Norgen's *Escherichia coli* (E.coli) O157:H7 Quantified Bacterial DNA Standards (STX1 and STX2 genes) are cloned fragments of the two Shiga-like toxin regions purified using Norgen's sample preparation technology. They include partial sequences of STX-1 subunit A gene and STX-2 subunit A gene. Please refer to Appendix A for sequence information. The purified DNA is quantified vigorously using multiple methods including spectrophotometry, gel densitometry and real-time PCR. It is intended to be used as positive controls or PCR quantification standards for *E. coli* O157:H7. This product is compatible with Norgen Biotek's *E. coli* O157:H7 TaqMan PCR Detection Kit (Cat. TM41350) and *E. coli* O157:H7 TaqMan PCR Detection Probe/Primer and Control Set (Cat. TM41310).

### **Specifications**

<b>Volume Provided</b>	250 µL (for <b>STX1</b> ) 250 µL (for <b>STX2</b> )
<b>DNA Quantity</b>	2 x 10 <sup>4</sup> copies per µL

### **Storage Conditions**

Upon receipt, store Norgen's *Escherichia coli* (E.coli) O157:H7 Quantified Bacterial DNA Standards (STX1 and STX2 genes) at -20°C or lower. Avoid multiple freeze-thaw cycles. If needed, prepare smaller working aliquots and store at -20°C or lower.

### **Quality Control**

In accordance with Norgen's ISO 9001 and ISO 13485-certified Quality Management System, each lot of Norgen's *Escherichia coli* (E.coli) O157:H7 Quantified Bacterial DNA Standards (STX1 and STX2 genes) are tested against predetermined specifications to ensure consistent product quality.

### **Product Use Limitations**

Norgen's *E. coli* O157:H7 Quantified Bacterial DNA Standards are designed for research purposes only. They are not intended for human or diagnostic use.

### **Safety Information**

Biosafety level 2 practices are recommended for work involving *E. coli* O157:H7. Ensure the appropriate containment equipment and facilities are used for activities involving cultures or potentially infectious clinical materials. Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working. Norgen's DNA purification technology has been shown to eliminate the infectivity of most infectious agents including viruses and bacteria. However, this product is not tested for infectivity and should be handled in accordance to Biosafety level 2 practices, any local safety guidelines or Good Laboratory Practices.

**Important Notes Prior to Beginning of Protocol:**

- Two separate tubes contain quantified DNA fragments of partial sequence of the Shiga-like toxin genes: STX1 and STX2. Either one (or both) of the standards could be used as the control template in a PCR reaction.
- The sequence of the STX genes covered by the quantified standard is provided in **Appendix A**. Please ensure that the PCR primers used are within the region described.

**1. Usage of *E. coli* O157:H7 Quantified Bacterial DNA Standards**

a. Thaw the original tubes provided or aliquots of the DNA standards on ice.

**Note:** Avoid multiple freeze-thaw cycle as it may affect copy number

b. If needed, make a dilution of the DNA standard using either nuclease-free molecular biology-grade water or TE.

**Note:** Use any diluted DNA promptly. It is not recommended to freeze and re-use any diluted DNA

c. Apply 1 – 5 µL of the DNA standard or any dilutions to a PCR reaction promptly.

**Appendix A: Sequences of *E. coli* O157:H7 Standards Provided**

**1) STX1 (348 bp, based on GenBank Accession No. AE005174)**

CAGTTAATGTGGTTGCGAAGGAATTTACCTTAGACTTCTCGACTGCAAAGACGTATGTAGATT  
CGCTGAATGTCATTTCGCTCTGCAATAGGTACTCCATTACAGACTATTTTCATCAGGAGGTACG  
TCTTTACTGATGATTGATAGTGGCACAGGGGATAATTTGTTTGCAGTTGATGTCAGAGGGAT  
AGATCCAGAGGAAGGGCGGTTTAATAATCTACGGCTTATTGTTGAACGAAATAATTTATATGT  
GACAGGATTTGTTAACAGGACAAATAATGTTTTTATCGCTTTGCTGATTTTTTCACATGTTACC  
TTTCCAGGTACAACAGCGGTTACATTGTCTGGTG

**2) STX2 (587 bp, based on GenBank Accession No. AE005174)**

ATCCTATTTCCCGGGAGTTTACGATAGACTTTTCGACCCAACAAAGTTATGTCTCTTCGTTAAA  
TAGTATACGGACAGAGATATCGACCCCTCTTGAACATATATCTCAGGGGACCACATCGGTGT  
CTGTTATTAACACACCCCACCGGGCAGTTATTTTGTCTGTGGATATACGAGGGCCTTGATGTC  
TATCAGGCGCGTTTTGACCATCTTCGTCTGATTATTGAGCAAATAATTTATATGTGGCCGGG  
TTCGTTAATACGGCAACAAATACTTTCTACCGTTTTTCAGATTTTACACATATATCAGTGCCCG  
GTGTGACAACGGTTTTCCATGACAACGGACAGCAGTTATACCACTCTGCAACGTGTCCGCAGC  
GCTGGAACGTTCCGGAATGCAAATCAGTCGTCACACTGGTTTTTCATCATATCTGGCGTTAA  
TGGAGTTCAGTGGTAATAACAATGACCAGAGATGCATCCAGAGCAGTTCTGCGTTTTGTCACT  
GTCACAGCAGAAGCCTTACGCTTCAGGCAGATACAGAGAGAATTTTCGTCAGGCACTGTCTG  
AAACTGCTCCTGTGTATACGATGACGC

**Technical Support**

Contact our Technical Support Team between the hours of 9:00 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362.

Technical support can also be obtained from our website ([www.norgenbiotek.com](http://www.norgenbiotek.com)) or through email at [techsupport@norgenbiotek.com](mailto:techsupport@norgenbiotek.com).

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