

***E. coli* O157:H7 Quantified Bacterial DNA Standards**

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 *E. coli* O157:H7 Quantified Bacterial DNA Standards 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

Volume Provided	250 µL (for STX1) 250 µL (for STX2)
DNA Quantity	2 x 10 ⁴ copies per µL

Storage Conditions

Upon receipt, store Norgen's *E. coli* O157:H7 Quantified Bacterial DNA Standards 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 *E. coli* O157:H7 Quantified Bacterial DNA Standards 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)

CAGTTAATGTGGTTGCGAAGGAATTTACCTTAGACTTCTCGACTGCAAAGACGTATGTAGATTCGCTGAAT
GTCATTCGCTCTGCAATAGGTACTCCATTACAGACTATTTTCATCAGGAGGTACGTCTTTACTGATGATTGA
TAGTGGCACAGGGGATAATTTGTTTGCAGTTGATGTGACAGGGATAGATCCAGAGGAAGGGCGGTTTAATA
ATCTACGGCTTATTGTTGAACGAAATAATTTATATGTGACAGGATTTGTTAACAGGACAAATAATGTTTTT
TATCGCTTTGCTGATTTTTTACATGTTACCTTTCCAGGTACAACAGCGGTTACATTGTCTGGTG

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

ATCCTATTCCCGGGAGTTTACGATAGACTTTTCGACCCAACAAAGTTATGTCTCTTCGTTAAATAGTATAC
GGACAGAGATATCGACCCCTCTTGAACATATATCTCAGGGGACCACATCGGTGTCTGTTATTAACCACACC
CCACCGGGCAGTTATTTTGTCTGTGGATATACGAGGGCTTGATGTCTATCAGGCGCGTTTTGACCATCTTCG
TCTGATTATTGAGCAAATAATTTATATGTGGCCGGGTTTCGTTAATACGGCAACAAATACTTTCTACCGTT
TTTCAGATTTTACACATATATCAGTGCCCGGTGTGACAACGGTTTTCCATGACAACGGACAGCAGTTATACC
ACTCTGCAACGTGTGCGCAGCGCTGGAACGTTCCGGAATGCAAATCAGTCGTCACACTCACTGGTTTTTCATCATA
TCTGGCGTTAATGGAGTTTCAAGTGGTAATACAATGACCAGAGATGCATCCAGAGCAGTTCTGCGTTTTGTCA
CTGTACAGCAGAAGCCTTACGCTTCAGGCAGATACAGAGAGAATTTTCGTCAGGCACTGTCTGAAACTGCT
CCTGTGTATACGATGACGC

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) or through email at techsupport@norgenbiotek.com.

3430 Schmon Parkway, Thorold, ON Canada L2V 4Y6
Phone: (905) 227-8848
Fax: (905) 227-1061
Toll Free in North America: 1-866-667-4362