MMS E. Coli O157:H7 Detection Assay (100 Test)

Product Manual

Version 1.0

This product is intended for research use only.

Contents

A. Introduction and Principles of the Test

This fourplex, probe-based real-time qPCR assay is intended for detection of all known strains of E. coli O157:H7 in a little less than 1 hour (with extracted DNA as the starting material). Based on the Bacteriological Analytical Manual – Chapter4a (US FDA), this assay targets a highly conserved, 106bp-long segment of the O157 plasmid, as well as a 109bp and a 83bp highly conserved segments of the shiga toxin 1 and shiga toxins 2 genes, respectively. This is a screening test for the presence of the O157 plasmid as well as the shiga toxins virulence factors, but further confirmation by the ISO16654:2001/Amd 2:2023 standard is needed.

Target	**wzy gene (O157)**	**Shiga Toxin 1 (Stx1)**	**Shiga Toxin 2 (Stx2)**	**IC (Internal Control)**
Detection Channel	FAM	ROX	Cy5	HEX

Due to the variety of possible input samples (food, animals, bacterial culture, etc.) a synthetic DNA spike-in is included in the kit to serve as the extraction internal control. This approach ensures that the extraction and amplification steps are accounted for, thus eliminating the possibility of false negatives due to erroneous extraction and/or amplification.

B. Intended Use

This kit is intended for detection of all known serovars of E. coli O157:H7 with extracted DNA as the starting material. The kit does *NOT* include reagents for DNA extraction. See (Specimen Preparation) section for our recommended DNA extraction kits for each type of biological specimen.

It should be noted that this kit is intended for research use only. Please do not use it for human medical diagnostics.

C. Kit Contents and Storage

The kit comes in a package of five tubes, as indicated in the following table. All components must be stored at -20°C. The MMS Oligo Mix should not be exposed to light for extended periods of time.

Component	Volume (uL)
qPCR Master Mix	1,000
MMS Oligo Mix	200
Negative Control	100
Positive Control	100
Extraction Internal Control (IC)	1,000

D. Required Materials That Are Not Included in the Kit

- Real-time PCR thermal cycler (see next section: Compatible Instruments)
- PCR plates compatible with your real-time PCR thermal cycler
- Optical adhesive seal and an applicator to seal off the PCR plate
- PCR plate microcentrifuge
- Pipettes and filtered pipette tips in the range of 1–1,000µL
- 1.5mL or 2.0mL microcentrifuge tubes
- Microcentrifuge with 1.5mL/2.0mL rotor
- (highly recommended) Ice or a cooled PCR stand/rack
- (optional) RNase-free water

E. Compatible Instruments

Our kit is compatible with commercially available open system real-time PCR thermal cyclers that can detect fluorescence at wavelengths 520nm (e.g. FAM) and 535nm (e.g. HEX, VIC, etc.). We have verified our assay on the following systems:

- 7500 Fast Real-Time PCR System (ABI, 4351106)
- QuantStudio™ 5 Real-Time PCR System (ABI, A28574)
- QuantStudio™ 7 Flex Real-Time PCR System (ABI, 4485688)
- CFX Opus 96 Real-Time PCR System (Bio-Rad, 12011319)

Please consult us about the compatibility of your thermal cycler by sending an email to support@havensci.com.

F. Good PCR Practices

Contamination is the worst enemy of PCR. Although the positive and negative controls in our kit can help in detecting contamination, dealing with contamination could prove troublesome and costly. Here, we list a few measures that could help you avoid contamination:

- Have physically separate workstation for PCR preparation, sample loading, and nucleic acid extraction. Each workstation should have its own dedicated pipettes and other small instruments, and should preferably be in a separate room.
- Always work under a cabinet (laminar flow for PCR preparation, and safety cabinet for nucleic acid extraction and sample loading).
- Use filtered tips. They are more expensive, but the small extra cost may save you valuable time, effort, and money down the line.
- If you need to analyze the PCR product after PCR cycling has finished, never unseal the plate near your PCR preparation or sample loading workstations. Even the smallest of aerosols could contain tens of millions of PCR templates.

G. Specimen Preparation

Make sure to have the extracted DNA ready before proceeding to PCR preparation in the next section. We recommend using the following DNA extraction kits depending on your biological specimen:

Extraction Technology	Extraction Kit	Suitable Sample Types
Magnetic beads	Mag Bacterial DNAbler Cat. No. DE92032 (Optimized on our MagEX 32 automated extraction system, Cat. No. NE032)	• **Pre-enriched food sample** • **Bacterial plates** • **Bacterial growth media**
Direct Extraction	DNAbler - Gram Negative Bacteria Direct Lysis Kit Cat. No. DE90100	
Silica columns	DNAbler - Cells and Tissue Cat. No. DE95050	• **Animal tissue** • **Bacterial plates** • **Bacterial growth media**
Silica columns	DNAbler – Blood Cat. No. DE96050	• **Animal blood**

H. Protocols

This kit includes an Extraction Internal Control (IC) to serve as a control for both the extraction and qPCR steps. The IC should be added to the lysis buffer-sample mixture during the extraction step. However, our DNAbler - Gram Negative Bacteria Direct Lysis Kit (Cat. No. DE90100) is not compatible with this protocol, and the IC should be mixed with the qPCR reaction master mix instead, serving as a control for the qPCR step only.

This section provides two protocols for extraction as follows:

- Standard protocol (page 9) → use it with any of our extraction kits listed in section H of this manual (Specimen Preparation, page 9)
- Direct lysis protocol (page 11) → use it only with our DNAbler - Gram Negative Bacteria Direct Lysis Kit (Cat. No. DE90100)

Standard protocol

1. Based on your sample type and desired extraction technology, extract DNA from each sample using one of our recommended kits in section H of this manual (Specimen Preparation, page 11). During extraction, mix 10µL of the Extraction Internal control (IC) provided in this kit with each sample ***AFTER*** adding the lysis buffer. The extracted nucleic acid should be used immediately after extraction (within 30 minutes) or stored at -20°C immediately after extraction.

2. Thaw all kit components at room temperature then place them on ice. Mix each tube by gentle flicking followed by a quick spin down to collect the tube's component to the bottom of the tube. ***Do NOT exceed 3 freeze-thaw cycles!***

3. Prepare a reaction master mix by mixing the following components (5% overage should be included to account for pipetting errors):

    a. *10 µL of qPCR Master Mix / reaction*
    b. *2 µL of the MMS Oligo Mix / reaction*

Example

Component	Per 1 reaction	X10 reactions (+5% overage)
qPCR Master Mix	*10 µL*	*105 µL*
MMS Oligo Mix	*2 µL*	*21 µL*

NOTE: Don't forget to figure in the positive and negative controls in your calculations!

4. Transfer 12 µL of the reaction master mix (prepared in step3) into each designated well on a 96-well optical qPCR plate.

5. Add 8 µL of each sample's extracted nucleic acid to its designated well; add 8 µL of the positive and negative controls to their designated wells.

NOTE: If you wish to add a smaller sample volume, fill to 20µL using RNase-free water

6. Seal the plate with optical cap strips or an optical adhesive seal. Spin down the plate to collect all components to the bottom of the plate.

7. On the Real-Time PCR thermal cycle software, define the detection filters as follows:

Target	O157	Stx1	Stx2	IC
Detection Channel	FAM	ROX	Cy5	HEX

8. On the software, define your Positive and Negative Controls.

9. Run the Real-Time PCR thermal cycler for 40 cycles as follows:

   Step1: *2 minutes - 95°C (initial denaturation)*

   Step2: *10 seconds - 95°C (denaturation)*

   Step3: *30 seconds - 60°C (annealing/extension)* → ***DATA COLLECTION***

   Step4: *go to step2 (X39 times)*

10. Analyze the results according to your thermal cycler's software. Use your judgement to set/modify the threshold of detection for each filter and/or omit amplification artifacts (e.g. non-sigmoidal amplification curve).

Direct lysis protocol

1. Extract DNA using our DNAbler - Gram Negative Bacteria Direct Lysis Kit (Cat. No. DE90100). There is no need to add the Extraction Internal control (IC) provided in this kit during extraction; however, it should be added to the reaction master mix (step3 below). The extracted nucleic acid should be used immediately after extraction (within 30 minutes) or stored at -20°C immediately after extraction.

2. Thaw all components at room temperature then place them on ice. Mix each tube by gentle flicking followed by a quick spin down to collect the tube's component to the bottom of the tube. ***Do NOT exceed 3 freeze-thaw cycles!***

3. Prepare the reaction master mix by mixing the following components (5% overage should be included to account for pipetting errors):

    a. *10 µL of qPCR Master Mix / reaction*
    b. *2 µL of the MMS Oligo Mix / reaction*
    c. *1µL of the IC/ reaction*

Example

Component	Per 1 reaction	X10 reactions (+5% overage)
qPCR Master Mix	*10 µL*	*105 µL*
MMS Oligo Mix	*2 µL*	*21 µL*
IC	*1 µL*	*10.5 µL*

NOTE: Don't forget to figure in the positive and negative controls in your calculations!

4. Transfer 13 µL of the reaction master mix (prepared in step3) into each designated well on a 96-well optical qPCR plate.

5. Add 8 µL of each sample's extracted nucleic acid to its designated well; add 8 µL of the positive and negative controls to their designated wells.

NOTE: If you wish to add a smaller sample volume, fill to 20µL using RNase-free water

6. Seal the plate with optical cap strips or an optical adhesive seal. Spin down the plate to collect all components to the bottom of the plate.

7. On the Real-Time PCR thermal cycle software, define the detection filters as follows:

Target	O157	Stx1	Stx2	IC
Detection Channel	FAM	ROX	Cy5	HEX

8. On the software, define your Positive and Negative Controls.

9. Run the Real-Time PCR thermal cycler for 40 cycles as follows:

   Step1: *2 minutes - 95°C (initial denaturation)*

   Step2: *10 seconds - 95°C (denaturation)*

   Step3: *30 seconds - 60°C (annealing/extension)* → ***DATA COLLECTION***

   Step4: *go to step2 (X39 times)*

10. Analyze the results according to your thermal cycler's software. Use your judgement to set/modify the threshold of detection for each filter and/or omit amplification artifacts (e.g. non-sigmoidal amplification curve).

I. Data Interpretation

Sample calling

For the three E. coli O157:H7-specific target, the acceptable cycle threshold of detection is Ct <40, which means that Ct ≥40 should be considered as non-detected. If all three E. coli O157:H7-specific targets were undetected, call the sample negative only if the Extraction Internal Control Ct is below 35. If it is greater than 35, there may have been an issue with the extraction, and we strongly recommend re-extracting the sample.

Detection of the wzy target alone indicates a potential positive E. coli O157:H7 infection. Detection of the wzy target with Stx1, Stx2, or both, indicates a potential positive infection with shiga toxin-producing E. coli O157:H7 (STEC O157:H7). In both cases, a confirmatory test may be needed. See section J of this manual (Confirmatory Testing).

Detection of Stx1, Stx2, or both, without wzy indicates a non E. coli O157:H7 shiga toxin-producing bacteria. This indicates a negative infection of E. coli O157:H7 and no further action is needed.

The following table summarizes our sample calling recommendations. A positive (+) sign indicates a Ct < 40 whereas a negative (–) sign indicates no amplification.

	WZY (FAM) CT	STX1 (ROX) CT	STX2 (CY5) CT	IC (HEX) CT	INTERPRETATION
CASE 1	+	–	–	Any	**Potential E. coli O157:H7 infection**
CASE 2	+	+	–	Any	**Potential shiga toxin-producing E. coli O157:H7 infection**
CASE 3	+	–	+	Any	**Potential shiga toxin-producing E. coli O157:H7 infection**
CASE 4	+	+	+	Any	**Potential shiga toxin-producing E. coli O157:H7 infection**
CASE 5	–	+	–	Any	**Non E. coli O157:H7 shiga toxin-producing bacteria**
CASE 6	–	–	+	Any	**Non E. coli O157:H7 shiga toxin-producing bacteria**
CASE 7	–	+	+	Any	**Non E. coli O157:H7 shiga toxin-producing bacteria**
CASE 8	–	–	–	≤35	**No infection**
CASE 9	–	–	–	>35	**Extraction failed; re-extract the sample**

Run validity

For the run to be valid, the Negative Control must return a negative infection result, whereas the Positive Control must return a positive infection result (see sample calling above). If not, the whole run is considered invalid.

J. Confirmatory Testing

A positive E. coli O157:H7 result of this assay indicates a potential E. coli O157:H7 infection, whether shiga toxins-producing or not. To confirm the result, follow the ISO 16654:2001/Amd 2:2023 standard protocol for E. coli O157:H7 identification.

K. Support

For questions, suggestions, or technical support, feel free to contact us by email on:

support@havensci.com

L. Quality Control

Each lot is tested against predetermined parameters to ensure consistent performance among all lots.