

MAST ISOPLEX® *E. coli* O157 kit

DNA/LYO4 20 tests

For use with the real-time thermal cyclers with FAM and TAMRA detection capacity.

Intended Purpose

MAST ISOPLEX® *E. coli* O157 kit is optimised for typing of O157 *Escherichia coli* from human stool samples with loop-mediated isothermal amplification (LAMP) using MAST ISOPLEX® Probe technology. The kit contains reagents for detection of Perosamine synthetase (RfbE) expressed by *E. coli* O157. *E. coli* O157 genes can be detected simultaneously in a duplex assay format with an inhibition control DNA (IC DNA).

Components of the kit

1. *E. coli* O157 LAMP Pellets (PEL2) x 2 pellets. Violet cap tubes.
2. *E. coli* O157 Primer and Probe mix with Inhibition Control DNA (PP2) x 2 tubes. Blue cap tubes
3. Positive Control DNA (O157) - Brown cap tube.
4. Reconstitution Buffer (RB) x 1 tube. Yellow Cap Tube.
5. Water, molecular grade (WTR) x 1 tube. Black Cap Tube.

Storage and Shelf life

Store the unopened kit at 2°C to 8°C away from direct sunlight until the expiry date shown on the pack label. Once reconstituted, all reagents should be aliquoted and stored at minus 20°C until the expiry date shown on the tube label. Store all other components at 2°C to 8°C away from direct sunlight.

Warnings and Precautions

Precautions should be taken to prevent contamination of reagents in the kit and samples. The kit is designed to be used by trained laboratory personnel only. Reaction tubes should be kept closed at all times following addition of reagents and discarded without opening following use, according to local health and safety guidelines. To avoid any contamination with the amplified product, never open a vial after amplification. Do not vortex reaction tubes. Ensure all reaction tubes are not scratched or cracked prior to use.

Materials required but not provided

1. DNA extracted from human stool sample to be provided by the user.
2. To ensure the best assay sensitivity it is recommended to use DNA extracts isolated from overnight cultures.
3. DNA from specimen sample should be obtained according to standard laboratory procedures.
4. Standard DNase free supplies such as assay reaction tubes, pipettes and pipette tips.
5. An instrument capable of performing isothermal incubation of reaction tubes at the desired temperature such as the Applied Biosystems (ABI) 7500 FAST REAL-TIME PCR system, equivalent in-house thermocycler. The equipment should have a

fluorescent reader with FAM and TAMRA detection channels for recognition of amplification products.

Preparation of reagents

Reconstitution of lyophilized components:
Spin tube briefly in a microcentrifuge and ensure the lyophilized reagents pellets are at the bottom of the tube. Reconstitute reagents as described in table 1. Mix gently by pipetting up and down several times. Each reagent tube is sufficient for 10 reactions.

Table 1: Reconstitution of lyophilized components

Component	Diluent	Volume
PEL2	RB	20µl
PP2	H ₂ O	10µl
O157 DNA	H ₂ O	20µl

O157 assay set-up

Use PEL2 pellets for O157 assays. Each reconstituted PEL2 pellet can be used for ten assay reactions (10 µl per assay). Set up the reaction as described in table 2.

Table 2: O157 assay set-up

O157 assay set-up	Per sample
PEL2 reconstituted in RB	2µl
Reconstituted PP2	1µl
DNA sample or O157 DNA (positive control)	1-7µl of DNA sample or 1µl of O157 PCDNA
WTR	0-6µl
Total reaction volume	10µl

For a negative control, prepare a no-template control (NTC) by replacing DNA sample with molecular grade water (WTR).

Programming Information for selected real-time thermal cycler

Dye selection:

TAMRA for detection of IC
FAM for detection of O157
Temperature: 63°C
Sample volume: 10µl
ROX (if available): unselected
Assay cut off time: 40 minutes

Data analysis:

Baseline: 3 to 5 cycles
Threshold set up: above the plot generated by the negative control

Interpretation of results

Thermocycler results: A positive result is indicated by presence of an amplification curve and a negative result is indicated by fluorescence without amplification within the reaction time as shown below on an ABI 7500 FAST REAL-TIME PCR system.

Figure 1: Example of amplification plots for a duplex assay

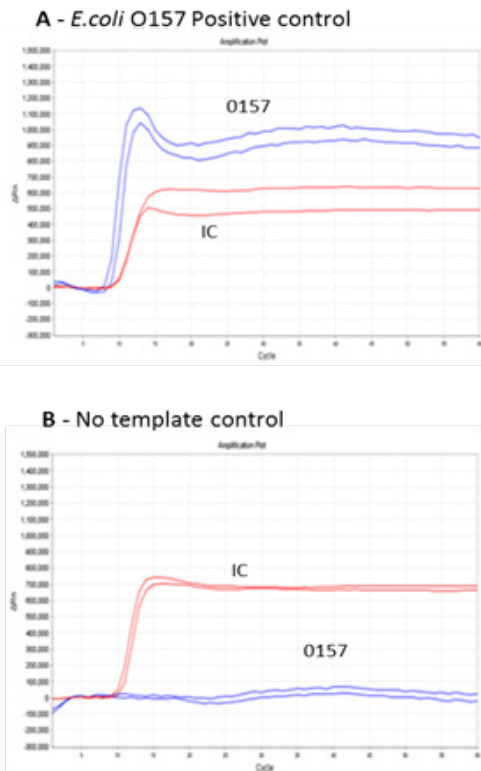


Table 3: Criteria for valid interpretation of LAMP multiplex assay with an inhibition control (IC)

Controls	Target gene (O157 FAM)	IC-TAMRA	Interpretation
Negative Control	-	+	Valid
Positive Control	+	+	Valid
Sample	-	+	Negative for the target gene
Sample	-	-	Invalid
Sample	+	+/- *	Positive for the target gene

* IC TAMRA can occasionally fail to amplify if target DNA is present in extremely high quantities, out-competing IC TAMRA primers and probes for reagents.

Quality control

It is recommended that quality control on MAST ISOPLEX® *E. coli* O157 kit is performed using the LAMP pellets (PEL2) Positive Control DNA (O157), the Probes, Primers and IC DNA mix (PP), pellet reconstitution buffer (RB) and the molecular grade water (WTR) supplied in the kit per test run. These tests will ensure the reagents perform as specified and no contamination of kit reagents has occurred. If control reactions give incorrect results, check for signs of deterioration or contamination of kit reagents and perform a retest. If retest also fails, contact Mast technical support department for advice.

Limitations of use

These products are for use in the amplification of DNA samples using standard DNA extraction methods. Quality Control will determine if kit reagents and controls are functional and free of contamination but cannot determine potential issues the user may experience with samples for DNA extraction. Sample extraction is the sole responsibility of the end user. Results obtained with this kit must be considered alongside other clinically relevant data when diagnosing an infection.

Specificity/Sensitivity/Limit of Detection (LOD)

Data obtained from DNA extracts from *E. coli* O157 presumptive faecal sample and negative culture (n=23).

Sensitivity – 100%

Specificity – 100%

LOD (Genome copy number) O157: 23582