WizDx™ CrystalMix E. coli O157:H7

REF DX1242
96 Tests

INTENDED USE
WizDx™ CrystalMix E. coli O157:H7 kit is a Real-time PCR test for the qualitative detection of *E. coli* O157:H7 in extracted DNA of cultured cells. WizDx™ CrystalMix E. coli O157:H7 kit is for research use only.

PRINCIPLES OF THE TEST
WizDx™ CrystalMix E. coli O157:H7 kit combines all reagents necessary for successful Real-time PCR in a convenient individual aliquot and lyophilized in an 8-strip qPCR tube. Real-time PCR technology utilizes polymerase chain reaction (PCR) for the amplification of specific target sequences and target-specific probes for the detection of *E. coli* O157:H7(Enterohemorrhagic *E. coli*; EHEC) Shiga toxin 1 (*stx1*) and Shiga toxin 2 (*stx2*) gene in extracted DNA of the cultured cell. The probes are labeled with a fluorescent reporter and quencher dyes. Probes specific for *E. coli* O157:H7 *stx1* gene are labeled with the fluorophore FAM and *E. coli* O157:H7 *stx2* gene are labeled with fluorophore HEX. The probe is specific for the Internal Positive Control (IPC) is labeled with the fluorophore Cy5 to monitor for PCR inhibition and to validate the quality of the sample and detection result.

KIT STORAGE AND STABILITY
- Store at 4 - 25°C, Do not freeze the CrystalMix.
- Expires 12 months from the date of manufacture
- Do not use it once the cone-shape mix shrinks as dot form. It is damaged by rehydration.

KIT CONTENTS

Component	Amount	Cap Color
E. coli O157:H7 CrystalMix	96 Tubes	
E. coli O157:H7 Positive Control*	1 vial	
Deionized sterile water*	1 vial	

* Before using the positive control, add 200µL of Deionized sterile water and dissolve sufficiently before use.
* Positive control & Deionized sterile water are shipped at room temperature. After receipt, please store in refrigerated or frozen.

REAGENT AND EQUIPMENT TO BE SUPPLIED BY THE USER
- Real-Time PCR System
- Micropipette & sterile pipette tips
- Vortex mixer & microcentrifuge
- Protective ware & disposable gloves

TEST SAMPLE
- Cultured cell

SAMPLE COLLECTION, STORAGE, AND TRANSPORTATION
- Collect samples in sterile tubes.
- Specimens can be extracted immediately or frozen at -20°C to -80°C.
- Transportation of clinical specimens must comply with local regulations for the transport of etiologic agents.

QUALITY CONTROL
In accordance with Wizbiosolutions Inc. ISO 13485-certified Quality Management System, each lot WizDx™ CrystalMix E. coli O157:H7 kit is tested against predetermined specifications to ensure consistent product quality.

WARNINGS AND PRECAUTION
- Carefully read this instruction before starting the procedure.
- For reaserch use only.
- For single use only. Do not reuse.
- Do not use any reagent after the expiration date.
- Always use sterile, filtered pipette tips.
- Always wear personal protective equipment (gloves and a mask) when handling biohazardous agents in compliance with national regulations.
- Take care of the handling of the specimen to minimize the risk of infection.
- Dispose of waste in compliance with national or regional regulations after the test.

PROTOCOL

[STEP 1] DNA Preparation

1) Boiling extraction method (Enrichment culture)
1. Transfer 1 mL of enrichment cells to the microtube.
2. Centrifuge for 10 minutes at 13,000 rpm and discard supernatant.
3. Resuspend the pellet with 500 µL of deionized sterile water.
4. Centrifuge for 10 minutes at 13,000 rpm and discard supernatant.
5. Resuspend the pellet with 100 µL of deionized sterile water.
6. In a heating block or water bath, 100°C for 10 minutes.
7. Centrifuge for 3 minutes at 13,000 rpm.
8. Transfer the supernatant to a new microtube and use it as a sample DNA.

2) Boiling extraction method (Isolation culture)
1. Take a typical colony and suspend it in 100 µL of deionized sterile water.
2. In a heating block or water bath, 100°C for 10 minutes.
3. Centrifuge for 3 minutes at 13,000 rpm.
4. Transfer the supernatant to a new microtube and use it as a sample.

3) Different brand DNA extraction kits are available.
For the DNA extraction, please follow the manufacturer's instructions.
The recommended extraction kits are as follows;
• WizPrep™ gDNA Mini Kit (Cell/Tissue) (REF. W71060, Wizbiosolutions Inc.)
• WizMag™ BMC DNA (REF. W7030, Wizbiosolutions Inc.)

[STEP 2] Prepare PCR Reaction

1) Prepare the PCR reaction as the following table.

Component	Sample	PC*	NTC*
E. coli O157:H7 CrystalMix	1 tube	1 tube	1 tube
Sample (DNA)	20 µL	-	-
E. coli O157:H7 Positive Control	-	20 µL	-
Deionized sterile water	-	-	20 µL
Total	20 µL	20 µL	20 µL

* **PC**: Positive control, **NTC**: Non-template control
☞ To avoid contamination, close the cap immediately after placing the sample in the tube.

2) Vortex for 10 - 30 sec. and briefly spin down for 5 sec.
3) Insert the CrystalMix tube into the Real-Time PCR System.

[STEP 3] Run Real-time PCR

1) Prepare the Real-time PCR program set-up as the following table.

Step	Temp.	Time	Cycle
UDG Treatment (Carryover prevention)	50 °C	120 sec.	1
Pre-Denaturation	95 °C	600 sec.	1
Denaturation	95 °C	15 sec.	40
Annealing (Probe detection)	60 °C	60 sec.	

2) Set up the threshold and baseline of the fluorescence probes as follows.

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PCR System	Fluorescence	Threshold	Baseline	
			Begin	End
CFX96™	FAM	100	Auto	
	HEX	100		
	Cy5	50		
CLEO™ Q16	FAM	Auto		
	HEX			
	Cy5			

[STEP 4] Analysis of Results

For interpretation, please refer to the Interpretation table.

• Cut-off value of sample or positive control : **Ct <40**

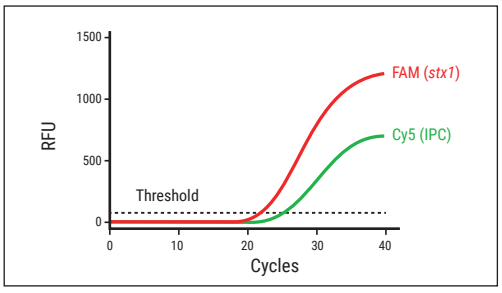
Test	FAM	HEX	Cy5	Results
#1	+	+	+/-*	*E. coli* O157:H7 *stx1/2* detected
#2	+	-	+/-*	*E. coli* O157:H7 *stx1* detected
#3	-	+	+/-*	*E. coli* O157:H7 *stx2* detected
#4	-	-	+	No detected
#5	-	-	-	Invalid (Retest)**

NOTE:

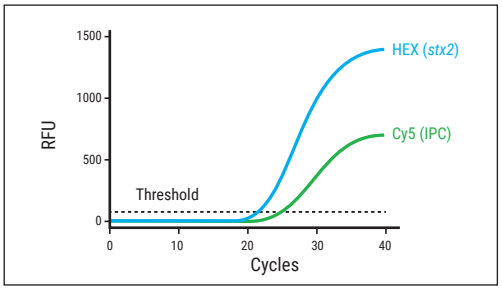
* Cy5 (Internal Positive Control; IPC) shall always be amplified. If the concentration of nucleic acids in the sample is high, the IPC signal may be inhibited and reduced or offset. In case the target gene signal is strong, Cy5 (Internal Positive Control; IPC) could be negative.
* If you want to check the IPC, it is recommended to dilute the sample and retest.
** If the result is determined as "Invalid", a retest shall be carried out. It is recommended to retest by extracting new specimens.

EXAMPLE OF THE RESULTS

E. coli O157:H7 *stx1* positive



E. coli O157:H7 *stx2* positive



LIMIT OF DETECTION (LOD)

To analyze the limit of detection (LoD) of WizDx™ CrystalMix E. coli O157:H7 kit, the DNA of *E. coli* O157:H7 (NCCP, 17170) were serially diluted (2.5X10¹ to 0.39x10⁰ copies/µL) and each concentration was repeated 24 times. Results were statistically analyzed by probit analysis. LoD is determined as the lowest DNA concentration that produced at least 95% positive results. The value of LoD is 5 copies/µL.

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TROUBLESHOOTING GUIDE

Observation	Possible Reason	
No signal increase is observed, even with positive controls	• Incorrect detection channel has been chosen.	• Set Channel settings to FAM, HEX (VIC), and Cy5.
	• Pipetting errors.	• Check for correct reaction setup. Repeat the PCR run.
	• No data acquisition programmed.	• Check the cycle programs.
Fluorescence intensity is too low	• Low initial amount of target DNA.	• Increase the amount of sample DNA. • Exchange all critical solutions.
Negative control samples are positive	• Carry-over contamination.	• Repeat the complete experiment with fresh aliquots of all reagents. • Always handle samples, kit components, and consumables in accordance with commonly accepted practices to prevent carry-over contamination. • Add positive controls after the sample and negative control reaction vessels have been sealed.
Fluorescence intensity varies	• Insufficient centrifugation of the PCR strips. Resuspended PCR mix is still in the upper part of the vessel.	• Centrifuge PCR strips.
	• Outer surface of the vessel or the seal is dirty (e.g., by direct skin contact).	• Always wear gloves when handling the vessels and seal.
Baseline drift phenomenon	• Invalid baseline setting applied.	• Settings → Baseline Setting → Apply fluorescence Drift Correction.

SYMBOL GLOSSARY

Symbol	Meaning	Symbol	Meaning	Symbol	Meaning
REF	Catalogue number		Manufacturer		Caution
LOT	Batch code	RUO	Research use only		Do not reuse
	Temperature limit		Consult instructions for use		Keep away from sunlight
	Use-by date	Σ	Contents sufficient for <n> tests		Keep dry
	Do not use if package is damaged				

ORDERING INFORMATION

Product	Cat No.	Package
WizDx™ CrystalMix E. coli O157:H7	DX1242	96 Test
WizMag™ BMC DNA	W7030	64 prep
WizPrep™ gDNA Mini Kit (Cell/Tissue)	W71060-100	100 prep
CLEO™ Q16 Real-time PCR system	CL0016	1 system
CLEO™ AP16 Nucleic Acid Extractor	CL2016	1 system

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