



LiliF™ VTEC Real-time PCR Kit

Distribuito in ITALIA da
Li StarFish S.r.l.
 Via Cavour, 35
 20063 Cernusco S/N (MI)
 telefono 02-92150794
 fax 02-92157285
 info@listarfish.it
 www.listarfish.it

BACKGROUND INFORMATION

VTEC is a particular group of the bacterium *Escherichia coli*. Although most strains of *E. coli* are harmless and live in the intestines of healthy humans and animals, VTEC strains produce a powerful toxin and can cause severe illness. In Ireland, the most common member of this group of bacteria is *E. coli* O157:H7, followed by *E. coli* O26. Other serogroups commonly linked to human illness in Europe include, O103, O145, O111 and O91.

LiliF™ VTEC Real-time PCR Kit is able to detect directly and specifically *E. coli* virus all serotype(H1 to H18) by real-time PCR technology on the basis of a genetic database of target nucleic acid fragments. Therefore, this kit can diagnose very sensitive, fast and accurately. The kit contains a specific primer and probe set for a highly conserved region based on current sequence alignments of all *E. coli* virus subtypes, allowing the RNA detection. It can determine the infecting all serotype and accurately and sensitivity detect multiple detection genes at one time using the real-time PCR (quantitative) method, and take only 2 hours for detection. Fast and sensitive detection of pathogen enables patients to get appropriate treatment and prevent the rapid spreading of disease by separating patients immediately.

PRINCIPLES

This product is a qualitative Real-time PCR testing product with 5' nuclease assay technology and CLP™ technology which provided flexibility in Tm (melting temperature) of primer design for optimization of reaction condition, and maximizes PCR specificity and sensitivity through the control of non-specific priming.

The assay is a real-time Real-time PCR that discriminates *E. coli* virus in one reaction. The assay is composed of two principal steps: (1) nucleic acid extraction from specimens, and (2) amplification of the target extracted nucleic acid fragment using fluorescent probe and specific primers pair. An internal control is used to monitor the extraction process and to detect Real-time PCR inhibition.

INTENDED USE

- For Research Use Only, Not for use in diagnostic procedures.
- This kit is developed, designed, and sold for research purpose only. It is not intended to be used for human or animal diagnosis of diseases. Prior to using it for other purposes, the user must validate the system in compliance with the applicable law, directives, and regulations.
- This product is research reagent of infectious disease for professional use to restrict the public use for animal diseases.

KIT CONTENTS

No	Contents	Composition
1	2X qPCR Master Mix	Real-time PCR Reaction solution < 0.01% Hot start Taq DNA Polymerase < 0.01% dATP, dTTP, dGTP, dCTP < 0.01% PCR additive materials
2	Detection Solution	<0.005% Primer/probe for VTEC
3	DNase/RNase Free Water	Ultrapure sterilized distilled water
4	Positive Control	< 0.001% Non-infectious plasmid DNA containing VTEC gene partial fragment sequences

RUO Research use only

REF IP24736



DESCRIPTION

- 2X qPCR Master Mix : Colorless and transparent liquid
- Detection Solution: colorless or pale pink liquid in dark brown micro tube
- Positive Control : Colorless and transparent liquid
- DNase/RNase Free Water : Colorless and transparent liquid

REAGENTS & CONSUMABLES TO BE SUPPLIED BY USER

- Real-time PCR Instrument (Line Gene 9600 plus : BIOER)
- Disposable gloves
- Pipettes & Sterile pipette tip (with filter)
- AutoXT bacteria gDNA Kit
- Table top centrifuge
- Vortex mixer
- Passive reference dye (Optional)

NOTICE BEFORE USE

※ Precautions before Testing

- All procedures must be done on a clean bench that should be cleaned with 70% alcohol or 10% household bleach (Na-hypochlorite) after use.
- The experimenter should wear a lab coat gloves, masks, etc., and to always be careful.
- The specimen used should be kept separate. If discarded, it is considered to be a biological hazardous substance after high-pressure sterilization and discarded.

※ Preparation, Preservation and Transportation of Specimen

- Specimen type
 - Food, Bacteria
 - Keep samples below -20°C before use, unless you have prepared them right before use.
- How to deliver and store the specimen
 - Store the specimen at 4°C with ice in the ice box and deliver it quickly to the test centers
 - Keep the specimen away from the ice to avoid freezing in the case of liquid sample
 - Store -20°C or -70 °C when the sample can not be delivered immediately
 - Store -20°C for a week
- Cautions of extraction and delivery of specimen
 - Wear the protection equipment before collecting the specimens
 - Deliver the specimen immediately under 4°C with a written request
 - Personal protection equipment : N-95 mask, gloves, safety goggles, bumper cap, overshoes
- Inappropriate specimen : The following aspects should be avoided to test
 - Samples against the standard of epidemiology or clinical diagnosis
 - Samples with inappropriate delivery temperature or incorrect container
 - Samples which is spoiled from the container

※ Preparation before Testing

- 2X qPCR Master Mix, Detection Solution, Positive control and etc, reagent
 - Please use at thawing temperature before starting the test. However, it is not recommended to leave at room temperature for more than 1 hour in thawing condition.
 - Repetition of continuous freezing and thawing can affect product performance. Although this product has confirmed that there is no abnormality in the refrigeration up to 50 times, it may cause deterioration of the performance of the product enzyme and other reagents if the cold decomposition is repeated irrespective of the use.
 - It is recommended that each thawed solution be placed on ice until just before use.
 - Thawed solutions should be mixed vortex lightly and spin down to homogeneous solution.
- Nucleic acid extraction of specimen

To extract the nucleic acid from the specimen, we recommend preparing the specimens and using AutoXT bacteria gDNA Kit.

※ Extraction of nucleic acid from specimen

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

- Prepare a buffer on a well plate as shown below.
- Columns 1 and 7: Lysis Buffer 500 µL + Proteinase K 20 µL
- Columns 2 and 8: Washing Buffer A 500 µL + RNase A 10 µL
- Columns 3, 4, 9 and 10: Washing Buffer B 500 µL
- Each 5th, 11th column: Elution Buffer 100µL
- 6 and 12 for each: Bead Solution 200 µL
- Add 200 µL of the yeast extract to be extracted to the 1st and 7th heat.
- Combine the Tip in the plunger, place the Deep Well block on the extraction device, and run AutoXT BAC.
- Be careful not to stop the protocol until the extraction is complete or not opening the front door.

※ Real time PCR Test phase

- Prepare a PCR tube for the real-time PCR reaction as many as the number of samples + 2.

⚠ Where +2 corresponds to positive control and negative control. In case of LineGene 9600 plus recommended by our company, it is possible to label on the cap of the tube, but if you use other equipment, please do not label the cap and be able to identify it by a separate method.

Contents	Sample	Positive Control	Negative Control
2X qPCR Master Mix	10 µL	10 µL	10 µL
Detection Solution	5 µL	5 µL	5 µL
Template	5 µL	-	-
Positive Control	-	5 µL	-
Sterile distilled water	-	-	5 µL
Total	20 µL	20 µL	20 µL

⚠ Since the positive and negative controls are intended to evaluate the effectiveness of the reaction, add one test to each test.

⚠ The negative control solution should be sterile distilled water provided in the product.

- Close the cap and mix so that the reaction mixture is evenly mixed. Then, remove the reaction liquid and air bubbles from the tube wall by spin-down.

⚠ Be careful not to shuffle the tubes in this procedure because real time PCR does not label the tubes separately.

Proceed with the PCR reaction according to the program set as follows.

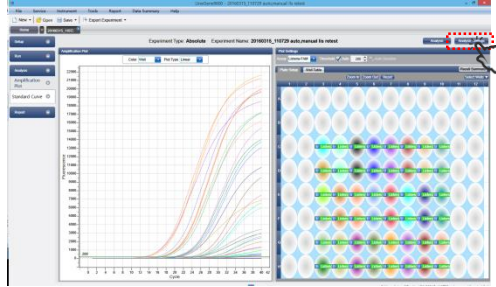
Cycle	Part	Temp	Time	Setting of fluorescence channel	
1	Initial Denaturation	95°C	5 min.	VT1	HEX
40	Denaturation	95°C	15 sec	VT2	FAM
	Annealing/Extension	55°C	30 sec	■ The gray notation indicates the signal detection interval	

ANALYSIS AND INTERPRETATION OF RESULTS

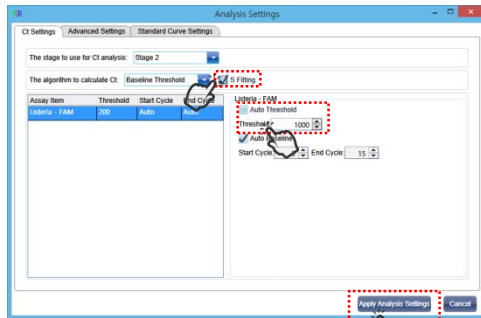
※ Setting analysis parameters

- After the experiment is finished, it is recommended to save the file and set the parameters of analysis by the following method. The following describes the parameter setting for LineGene 9600 plus. If you use other equipment, you should set it based on the manual and results of each equipment manufacturer.

- Select "Analysis Settings" at the top right of the analysis screen.



- When the Analysis Settings window opens, make the following settings.



- Set the S fitting check box, clear the Auto Threshold check box, and enter a Threshold value of 160. Auto Baseline keeps checked. If you select "Apply Analysis Settings" afterwards, analysis will be done.

Target	Dye(Fluorophore)	Threshold	Baseline	S fitting
VT1	HEX	80	Auto	check
VT2	FAM	60		

- If you select "Result Summary" afterwards, you can check the Ct value of each analyzed well simultaneously.



※ Interpretation of result

- It is recommended that the negative control be judged first and the result judged for the sample when the negative judgment appears.
- The validity of the PCR reaction itself can be verified using the positive control provided.
- Example of result judgment

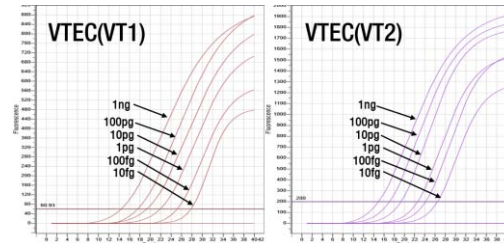
Contents	Fluorescent channel		Result
	HEX	FAM	
Positive Control	< 38		Effective
Positive Control	38 ≤		Invalid
Negative Control	38 ≤		effective
Negative Control	< 38		Invalid
Sample 1	38 ≤		Negative
Sample 2	< 38		Salmonella Positive

1. Quality Control

This product contains a positive control in the product. Therefore, in order for the user to judge whether the performance of this product is working properly, please check whether the positive result and the negative reference solution react with each other and whether the result is normal. If the proper storage environment and unusual results within the life of the product are obtained, the manufacturer may be asked to exchange the product.

EXAMPLE RESULTS

※ Sensitivity testing of LiliF™ VTEC Real-time PCR Kit



Seven steps of decimal dilution from the gDNA extracted from E.Coli O157 the culture fluid were detected positive by PCR.

CAUTIONS

- All procedures must be done on a clean bench and clean bench should be wiped clean with alcohol after use.
- Always wear protective gear during handling chemical materials and the test should be handled by professionally trained person.
- The every specimen may have the risk of infection and unknown diseases, so be careful when handling them to prevent infection by users and indirect contacts.
- Do not mix reagents from different lots of this product.
- Carefully treat the reagents and specimens of this product to prevent the aerosol from splashing when opening the lid of the container and prevent reagents and specimens from splashing by wearing a mask.
- Be careful not to spill the aerosol when you open the container lid by carefully handling the reagents and samples of this product. In addition, wear a mask to prevent reagents and specimens from splashing on your mouth.
- During the handling of this product and specimens, do not place any instruments that may hurt you, such as needles or knives, and avoid safety accidents by not using such instruments.
- When using allantoic fluid or serum as a specimen, be careful about coagulation and keep low temperature (4 °C) in the experiment.
- If you want to dispose of suspected specimens, contaminated test materials and instruments, disinfect them using high pressure steam sterilization or disinfection. If you want to disinfect, treat with 70% ethanol, 10% bleach solution for 10 ~ 30 minutes .
- Because the optical tube of Real time PCR which is used according to each equipment is different, this product can be supplied in a customized form quickly without stock. Therefore, before ordering, please check the compatible profile of the tube and the model of the equipment you are using and contact us.
- All reagents contained in the kit should be stored at -20°C.
- Be aware of contamination or direct contact from test specimens.
- For more accurate testing, we recommend using samples taken within 5 days after symptoms.
- To prevent contamination, we recommend that you observe the following :
 - It is advisable to separate extraction space and PCR space so that they do not overlap.
 - Centrifuge, test bench and pipette should be periodically cleaned with Bleach solution (10% house-hold bleach) to prevent the entry of unknown contaminants.
 - Treat the reaction solution in the order of negative control (NTC) → sample → positive control during PCR reaction.

PACKAGING & STORAGE METHOD

No	Contents	50 Test / Kit	Temp
1	2X qPCR Master Mix Solution	280 µl x 2 vial	-25°C ~ -15°C
2	Detection Solution	140 µl x 2 vial	-25°C ~ -15°C
3	Positive Control	25 µl x 3 vials	-25°C ~ -15°C
4	DNase/RNase Free Water (Negative Control)	1 ml x 1 vial	-25°C ~ -15°C
5	Manual	1 ea	-25°C ~ -15°C

SHELF LIFE

This product is 12 months from date of manufacture when unopened, and 6 months after opening.

- All reagents of the kit should be kept frozen (-25 °C ~ -15 °C) according to the storage method, Should be used within.
- Freezing reagents should be at room temperature (15 ~ 25 °C) and should be frozen immediately after use.



EXPLANATION OF SYMBOLS

