LiliF™ Listeria Real-time PCR Kit

RUO Research use only REF IP24386 Σ 50 -20℃

BACKGROUND INFORMATION

Listeria is a genus of bacteria that, until 1992, contained 10 known species, each containing two subspecies. As of 2014, another five species were identified. Named after the British pioneer of sterile surgery Joseph Lister, the genus received its current name in 1940. Listeria species are gram-positive, rod-shaped, and facultatively anaerobic, and do not produce endospores. The major human pathogen in the genus Listeria is L. monocytogenes. It is usually the causative agent of the relatively rare bacterial disease listeriosis, an infection caused by eating food contaminated with the bacteria. Listeriosis can cause serious illness in pregnant women, newborns, adults with weakened immune systems and the elderly, and may cause gastroenteritis in others who have been severely infected.

This LiliF™ Listeria Real-time PCR Kit is designed as a probe-based real-time PCR product targeting the specific sequence in the hly gene of Listeria monocytogenes. It uses the highly efficient Hot Start PCR Enzyme to maximize the detection performance of the product. This product is suitable for testing the mixed Listeria monocytogenes bacteria in the specimens with high sensitivity and specificity.

PRINCIPLE

- LiliF™ Listeria Real-time PCR Kit is a 5 'Nuclease Assay Real-time PCR product.
- This product specifically tests Listeria monocytogenes for DNA from food-borne samples and microorganism culture. The validity of the test can be verified by providing Control DNA for the verification of the reaction.
- The target gene is the hly gene of Listeria monocytogenes. It can specifically detect only Listeria monocytogenes among other Listeria.

INTENDED USE

This product is a kit for the detection of harmful microorganisms in various foods and livestock products. It is a product for qualitative analysis by real-time PCR test for the presence or absence of Listeria monocytogenes in microorganism culture and microbial culture.

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 hly
3	DNase/RNase Free Water	Ultrapure sterilized distilled water
4	Positive Control	< 0.001% Non-infectious plasmid DNA containing hly gene (from L. monocytogenes) partial fragment sequences

DESCRIPTION

1. 2X qPCR Master Mix : Colorless and transparent liquid
2. Detection Solution: colorless or pale pink liquid in dark brown micro tube
3. Positive Control : Colorless and transparent liquid
4. 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

1) Specimen type
   - Food, Bacteria
   - Keep samples below - 20℃ before use, unless you have prepared them right before use.

2) How to deliver and store the specimen
   - Store the specimen at 4℃ 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℃ or -70 ℃ when the sample can not be delivered immediately
   - Store -20℃ for a week

3) Cautions of extraction and delivery of specimen
   - Wear the protection equipment before collecting the specimens
   - Deliver the specimen immediately under 4℃ with a written request
   - Personal protection equipment : N-95 mask, gloves, safety goggles, bumper cap, overshoes

4) 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

1. 2X qPCR Master Mix, Detection Solution, Positive control and etc, reagent

1) 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.

2) 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.

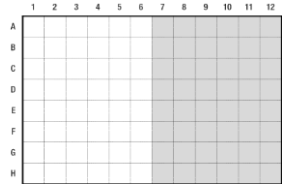
3) It is recommended that each thawed solution be placed on ice until just before use.

4) Thawed solutions should be mixed vortex lightly and spin down to homogeneous solution.

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

※ Real time PCR Test phase

1. 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.

2. 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
1	Initial Denaturation	95℃	5 min.
40	Denaturation	95℃	15 sec
	Annealing/Extension	55℃	30 sec

Setting of fluorescence channel	
Listeria	FAM

☛ The gray notation indicates the signal detection interval

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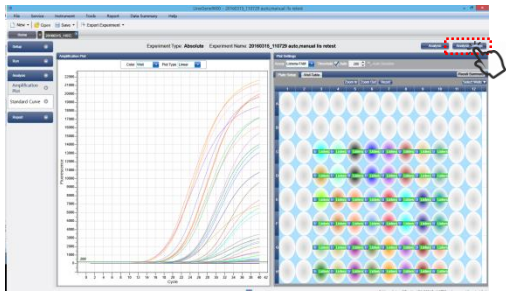
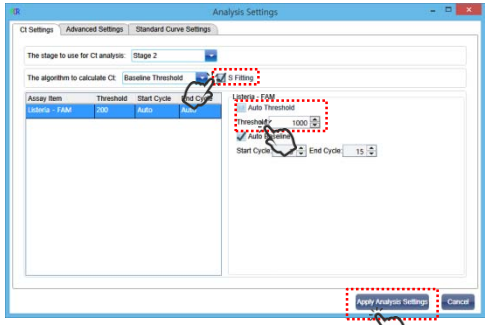
ANALYSIS AND INTERPRETATION OF RESULTS

※ 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.



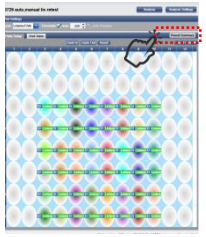
- When the Analysis Settings window opens, make the following settings.



- 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
hly	FAM	1000	Auto	check

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



※ Interpretation of result

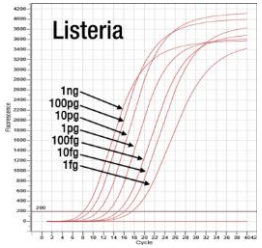
1) It is recommended that the negative control be judged first and the result judged for the sample when the negative judgment appears.
2) The validity of the PCR reaction itself can be verified using the positive control provided.
3) Example of result judgment

Contents	Fluorescent channel	Result
	FAM	
Positive Control	< 38	Effective
Positive Control	38 ≤	Invalid
Negative Control	38 ≤	effective
Negative Control	< 38	Invalid
Sample 1	38 ≤	Negative
Sample 2	< 38	Listeria 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™ Listeria Real-time PCR Kit



It was confirmed that the plasmid DNA containing the target gene (hly gene) in Listeria monocytogenes was 7-step decidual diluted and positive in PCR.

CAUTIONS

1. All procedures must be done on a clean bench and clean bench should be wiped clean with alcohol after use.
2. Always wear protective gear during handling chemical materials and the test should be handled by professionally trained person.
3. 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.
4. Do not mix reagents from different lots of this product.
5. 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.
6. 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.
7. 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.
8. When using allantoic fluid or serum as a specimen, be careful about coagulation and keep low temperature (4 ℃) in the experiment.
9. 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 .
10. 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.
11. All reagents contained in the kit should be stored at -20℃.
12. Be aware of contamination or direct contact from test specimens.
13. For more accurate testing, we recommend using samples taken within 5 days after symptoms.
14. 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℃~-15℃
2	Detection Solution	140 µl x 2 vial	-25℃~-15℃
3	Positive Control	25 µl x 3 vials	-25℃~-15℃
4	DNase/RNase Free Water (Negative Control)	1 ml x 1 vial	-25℃~-15℃
5	Manual	1 ea	-25℃~-15℃

SHELF LIFE

This product is 12 months from date of manufacture when unopened, and 6 months after opening.
1) All reagents of the kit should be kept frozen (-25 ℃ ~ -15 ℃) according to the storage method, Should be used within.
2) Freezing reagents should be at room temperature (15 ~ 25 ℃) and should be frozen immediately after use.

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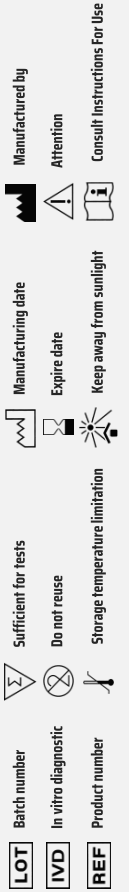
EXPLANATION OF SYMBOLS



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