LiliF™ HALAL Real-time PCR Kit (Pork)

RUO Research Use Only REF IPH54246 Σ 48 -20℃

BACKGROUND INFORMATION

To assure a high level of food and feed safety, accurate animal species identification and the detection of adulterants are two of the greatest challenges facing food and feed products companies today. Therefore, the need for scientifically valid species identity methods is increasingly important. Although a number of traditional morphological, microscopic and chemical methods have commonly been used for species identity testing, technologies using DNA offer reliable alternative methods that can provide increased precision in differentiating closely related species, as well as identifying intentional and accidental adulterants and contaminants.

LiliF™ HALAL Real-time PCR Kit (Pork) is designed for use by food and feed producers, dairies, marketers of these products, as well as regulators and auditors of final food and feed quality and safety. It is also intended to be used to verify that ruminant feed and feed supplements are properly labeled and do not contain ruminant materials.

PRINCIPLES

- The real-time PCR(polymerase chain reaction) DNA amplification technology exibits high sensitivity and specificity for direct detection of target gene. iNtRON developed a novel platform technique about primer design called CLP™ (complementary locking primer) 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 PCR (or qPCR) that discriminates cytochrome b gene in one reaction. The assay is composed of two principal steps:

  (1) Extraction of total DNA from meat(or food) samples,

  (2) The LiliF™ HALAL Real-time PCR Kit (Pork) is a qualitative Duplex real-time PCR test, for the detection of porcine specific gene and the Exogenous Internal Positive Control (IC) using specific primers and probes labeled with the fluorescent dyes. The target sequences are detected through the FAM and HEX (VIC) channel respectively. The primer and probe mixture provided exploits the so-called 5' Nuclease assay principle. During PCR amplification, forward and reverse primers hybridize to the target DNA. A probe is included in the same reaction mixture which consists of an oligonucleotide labeled with a 5'-reporter dye and a downstream 3'-quencher. During PCR amplification, the probe is cleaved and the reporter dye and quencher are separated. The resulting increase in fluorescence can be detected on a range of real-time PCR platforms. An internal control is used to monitor the extraction process and to detect PCR inhibition

INTENDED USE

The LiliF™ HALAL Real-time PCR Kit (Pork) is qualitative in vitro test for detection of Pork specific gene from all types of meat (or food) samples.

REQUIREMENTS INSTRUMENT

- Real-time PCR Instrument
- Pipettes
- Sterile pipette tip (with filter)
- Disposable gloves
- Miracle-AutoXT (Nucleic Acid Extraction System) or G-spin Total kit
- Centrifuge for micro-centrifuge tubes
- Vortex mixer
- Passive reference dye (Optional)

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DESCRIPTION

1. Pork detection PreMix : Colorless and transparent pellet in PCR Strip
2. Positive Control : Colorless and transparent liquid
3. DNase/RNase Free Water : Colorless and transparent liquid

KIT CONTENTS

No	Contents	Composition
1	Pork detection PreMix	< 0.01% Hot start Taq DNA Polymerase < 0.01% dATP, dTTP, dGTP, dCTP < 0.005% Pork cytb gene Primers/Probe
2	Positive Control	< 0.001% Non-infectious plasmid DNA(microbial) containing Pork cytb gene partial fragment sequences
3	DNase/RNase Free Water	No template control < DNase/RNase Free Water

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 samples 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. Sample type
   - Various food source sample, environmental sample, clinical material and raw meat materials are routinely examined.
   - The test sample should be stored 4℃ ~ -20℃ prior to use.
2. How to deliver and store the food or ruminant materials sample
   - Store the sample at 4℃ with ice in the ice box and deliver it quickly to the test centers
   - Keep the sample away from the ice to avoid freezing in the case of liquid sample
   - Store -20℃ when the sample can not be delivered immediately
   - Store -20℃ for a month
3. Cautions of extraction and delivery of samples
   - Wear the protection equipment before collecting the specimens
   - The specimen is needed to pack in stages to protect breakdown(3 steps are basically recommended)
   - Deliver the specimen immediately under 4℃ with a written request
   - Personal protection equipment : mask, gloves, safety goggles, bumper cap , protective overshoes

※ Preparation before Testing

1. Preparation of Reagents

   1) Pork detection PreMix
      - Leave it immediately on ice before use.
      - Do not leave it at room temperature more than 1 hour, after.

   2) DNase/RNase Free Water and Positive Control
      - Leave it at 4℃ for thawing.
      - Do not leave it at room temperature more than 1 hour.

2. Pretreatment of Samples.

   Total DNA extraction Kit should be used for nucleic acid extraction and the extraction steps should be followed by the protocol of each kit. : the kit explains the extraction method by Auto XT Bacteria gDNA Kit. (Autoprep type, iNtRON# 17169) or G-spin Total DNA Extraction kit (Spin column type, iNtRON#17045, below protocol)

   1. Slice off the prepared sample to suitable size by the scalpel or scissor.
   2. Approximately 50 mg of ground meat or food sample, and then transfer into 1.5 ml tube using a spatula.
   3. Add 200 µl Buffer CL, 20 µl Proteinase K and 5 µl RNase A Solution into sample tube and mix by vortexing vigorously.
   4. Incubate the lysate at 56℃ (preheated heat block or water bath) for 10 ~ 30 min.
   5. After lysis completely, add 200 µl of Buffer BL into upper sample tube and mix thoroughly. Then incubate the mixture at 70℃ for 5min.
   6. Centrifuge the sample tube at 13,000 rpm for 5 min to remove un-lysed tissue particles. Then carefully transfer 350 ~ 400 µl of the supernatant into a new 1.5 ml tube (not provided).
   7. Add 200 µl of absolute ethanol into the lysate, and mix well by gently inverting 5 - 6 times or by pipetting. DO NOT vortex. After mixing, briefly centrifuge the 1.5 ml tube to remove drops from inside of the lid.
   8. Carefully apply the mixture from step 6 to the Spin Column (in a 2 ml Collection Tube) without wetting the rim, close the cap, and centrifuge at 13,000 rpm for 1 min. Discard the filtrate and place the Spin Column in a 2 ml Collection Tube (reuse).
   9. Add 700 µl of Buffer WA to column and centrifuge for 1min at 13,000rpm.
   10. Add 700 µl of Buffer WB to the Column without wetting the rim, and centrifuge for 1 min at 13,000 rpm. Discard the flow-through and place the Column into a 2.0 ml Collection Tube (reuse), Then again centrifuge for additionally 1 min to dry the membrane. Discard the flow-through and Collection Tube altogether.
   11. Place the Spin Column into a new 1.5 ml tube (not supplied), and 30 - 100 µl of Buffer CE directly onto the membrane. Incubate for 1 min at room temperature and then centrifuge for 1 min at 13,000 rpm to elute.

STORAGE CONDITION

1. Store all component at ambient temperature(1~30℃) or below -25℃ ~ -15℃ according to the instruction within the expiry date given on the pack.
2. Frozen contents should be warmed at room temperature(15 ~ 25℃) or 4℃. After usage, these reagents have to be kept in a freezer immediately.
3. Instruction for storage condition and shelf-life of opened reagents.
   Note : Do not leave Pork detection PreMix at room temperature more than 1 hour after add water. Leave it at 4℃ or room temperature for thawing in dark place while protected from the light.

No.	contents	Storage conditions	Shelf-Life
1	Pork detection PreMix	frozen below -20℃	Within 6 months after opening, within expiry date of the kit
2	Positive Control	frozen below -20℃	Within 6 months after opening, within expiry date of the kit
3	DNase/RNase Free Water	frozen below -20℃	-

※ Testing Protocols

1. Place the appropriate number of strip tubes (one tube for each sample).
   ⚠ The use of ROX dye is necessary for instruments from Applied Biosystems and is optional for instruments from Agilent. Normalization is necessary to correct for fluorescent fluctuations due to changes in concentration or volume from instruments. (ABI 7500: 50nM ROX).
2. Add 15μl of Water to each of strip tubes.
3. Add 5μl of DNA sample to each of strip tubes.
4. For positive and negative confirmation, use 5μl of positive control or DNase/RNase Free water as a test sample.
5. Close the tube firmly, then Mix by vortex mixer.
   ⚠ If there is no vortexing the tube, there will be a bad signal baseline.
   ⚠ The result will be abnormal because of the rise of baseline, in case cap is not sealed firmly.
6. Spin down the tube by centrifugation, then put them into a real-time PCR cycler and process reaction following below table.

PCR Program (2 step)

Step	Cycle	Temp	Time
Pre-heat step	1	95 ℃	10 min.
PCR and Signal Detection	35	95 ℃	10 sec.
		64 ℃	**20 sec.**

Channel setting	
Pork	FAM
IPC	HEX

☛ Under line means Signal detection step

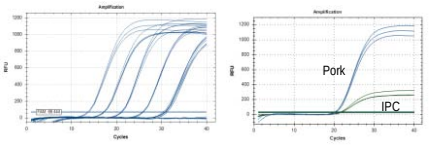
MEASUREMENT OF REAL-TIME PCR RESULT

※Interpretation of result

1. Results analysis
   1) It is recommended to refer to the manual of the relevant instrument because the analysis method differs according to the Real-time PCR machine used.
   2) It is advisable to judge the negative control first and proceed with the result judgment on the sample when it appears as a negative judgment.
   3) Using a positive control that is provided, it can verify the validity of the PCR reaction itself.
   4) The criteria for interpreting the results are as follows.
      - Manual Baseline : Baseline settings from 3 to 12 cycles
      - Threshold setting This value can be different for each device.
      - Most of them are set to Automatic but can be adjusted based on initial or NTC signal of amplification curve if necessary.
      - Ct cut off value : The values obtained after 34 cycles are excluded from the results
2. 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



10^5 ····· 10^1 copy

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Interpretation of Results

	Positive control	Negative control	FAM Target	HEX (VIC) IC	Interpretation
Case 1	+	-	+	+	The pork gene is detected in a sample.
Case 2	+	-	+	-*	The pork gene is detected in a sample.
Case 3	+	-	-	+	The pork target gene is not detected.
Case 4	+	-	-	-	Invalid result/retest
Case 5	+	+	+/-	+/-	
Case 6	-	+	+/-	+/-	
Case 7	-	-	+/-	+/-	

* Detection of the Internal Amplification Control in the respective channel is not required for positive result. A high copy number of target gene can lead to reduced or absent Internal Amplification Control signal.

TROUBLESHOOTING

Situation	Possible cause	Recommendation
Negative control samples are positive.	Carry-over contamination	• Exchange all critical solutions. • Repeat the analysis of all tests with fresh aliquots of all reagents. • Take measures to detect and eliminate the source of contamination.
No signal is detected for positive controls of amplification.	Incorrect programming of the real-time PCR instrument. The kit reagents have expired.	The PCR should be repeated after check for programming of instruments, storage conditions and the expiration date.
	The storage conditions for kit components have not complied with manufacturer instruction	
	Incorrect PCR reaction • Pipetting errors • Omitted reagents	The PCR should be repeated after check for correct pipetting scheme and reaction setup.
No signal is detected for IC on HEX (VIC) channel and porcine specific gene on FAM channel.	PCR inhibitors are present at a high concentration.	DNA extraction should be repeated.

CAUTIONS

1. The test samples are handled under the condition of unknown level (concentration), so the laboratory contamination is expected. Therefore, all glasses used for experiments must be sterilized and secure the personal safety.
2. Always wear protective gear during handling chemical materials and the test should be handled by professionally trained person.
3. To prevent infection owing to indirect contacts, always begin and perform test on a plastic mat or an underlay corresponding to this and after completion of test, burn it or discard it after it as biohazards waste heating at 120℃ for 20 ~ 30 minutes.
4. Positive control and sample should be added in PCR mixture tube at the separate place.
5. Centrifuge and pipette should be regularly sterilized by 10% bleach solution.
6. Do not use this lot together with another lot.
7. Don't pipette any of the kit reagents with mouth.
8. Smoking and ingestion of food or drink should be avoided while handling the kit reagents and samples.
9. All the waste should be sterilized before discarding.
10. Use the G-spin Total extraction kit for DNA extraction and recommend Auto XT (iNtRON. Cat. 17154).
11. The contamination should be considered very seriously. The work station should be kept with extreme cleanness not to have false-positive. Use RNase WiPER (iNtRON. Cat. 21131) to clean the desk or 1/20 diluted household bleach can be used alternatively.
12. Store the kit at -20℃.

PACKAGING INFORMATION

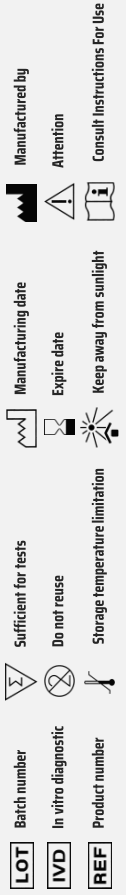
No	Contents	48 Test / kit
1	Pork detection PreMix	48 tubes
2	Positive Control	25 μl x 3 tubes
3	DNase/RNase Free Water	1 ml x 1 tube

SHELF-LIFE

- 12 month from manufacturing date
- Within 6 months after opening, within expiry date of the kit.

LiliF Diagnostics

EXPLANATION OF SYMBOLS



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