Pig DNA Real Time PCR Detection Kit IFU

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Intended Use

This kit is used for qualitative detection of Pig nucleic acid in various food and meat products. In view of the adulteration of meat products in the market, identify whether the meat products contain ingredients from Pig.

Principle

Polymerase chain reaction (PCR) method combined with Taqman probe was selected for detect pig nucleic acid.

Ingredients

Product Number	BSB46S1	BSB46M1
Components	**24T**	**48T**
Pig PCR Buffer	1 Tube (300 μL)	1 Tube (600 μL)
Primer /Probe Mix	1 Tube (180 μL)	1 Tube (360 μL)
Negative control	1 Tube (300 μL)	1 Tube (300 μL)
Positive control	1 Tube (300 μL)	1 Tube (300 μL)

Storage and period of validity

1. The kit need to be transported under freezing conditions.
2. The kit should be stored at -15℃ ~ -25℃ away from light, and avoid repeated freeze-thaw more than 5 times.
3. The kit can be stored for up to 12 months if all components are kept in the manner above. (Please use the kit in the period of validity).

Applied instruments

Line-Gene and Quant-Gene Series Real-time PCR detection system from Bioer, and the similar series Real-time PCR detection system of other companies.

Sample request

1. All kinds of raw and cooked meat processed products (including cans and ham sausages, etc.).
2. The samples should be placed in sterile and non-polluting centrifuge tubes to avoid cross-contamination; if not extracted or used immediately, they should be placed in a -80℃ refrigerator.

Using of the kit

1. Nucleic acid extraction

Samples can be extracted using the Biospin Genomic DNA Extraction Kit (BSC39) from Bioer.

2. Amplification reagent preparation

Thaw out the reagents at room temperature. Mix gently and centrifuge all reagents for a few seconds. Prepare PCR reagents according to the number of samples and controls as below:

Reagent	Pig PCR Buffer	Probe /Primer Mix
Dosage / test	12.5 μL	7.5 μL

After mixing PCR reagents above, distribute it into 0.2mL PCR tubes with 20μL per tube. Add 5μL the extracted samples or controls into PCR tubes above. Tighten the tube cover, remove bubbles by centrifugation, and then conduct PCR reaction.

3. PCR reaction

Set reaction procedure as following:

95°C 3 min
95°C 5 sec
60°C 10 sec
} 45 Cycles

Select the FAM channel of instrument for fluorescent signal collection. Instrument set fluorescent signals detecting at 60°C, reagent volume is 25 μL.

Quality control standards

	Result	Interpretation **of Test Results**
Positive Control	Ct value ≤ 35	All conditions are met in the same experiment, indicating that the experiment is valid, otherwise it is invalid.
Negative Control	No Ct value	

Result Analysis and Judgments

Result	**Result Judgment**
Ct value ≤ 40	Positive
Ct value>40	The sample needs to be re-tested. If the result is consistent with before or accord with positive judge standard, judge is positive, otherwise judge is negative.
No Ct value	Negative

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Note

1. Please read this manual carefully before experiment.
2. The whole process should be carried out in different area. Area I for preparing amplification reagent, area II for processing of tested samples, and area III for PCR amplification detection. All the articles in each district are for special use which cannot allow to be exchanged for avoiding pollution. The workbench should be cleaned immediately after the completion of each experiment.
3. Operators must take training before operation.
4. Biological Safety Cabinet should be used to ensure safety and prevent contamination. Harmful and toxic specimens and reagents in the experiment should be properly placed and kept by special custody; waste should be placed in special containers for proper disposal. The operating table and instruments should be wiped and disinfected with 1.0% sodium hypochlorite and/or 70% ethanol frequently. The experimental room and ultra-clean workbench should be treated with ultraviolet light regularly and after each experiment.
5. Mix and centrifuge well before using the reagents. Please use disposable PE gloves or rubber gloves during the whole operation; don’t open the tube after amplification, discard it into appointed containers.
6. Please make sure the reagent is within period of validity. Do not mix reagents from different batches.

Information of Manufacturer

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