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.

Product performance index

The kit can detect 0.1% of bovine-derived components.

Information of Manufacturer

Manufacturer: Hangzhou Bioer Technology Co., Ltd.
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Bovine DNA Real Time PCR Detection Kit

TECHNICAL SUPPORT:

For technical support, please dial phone number ： 0086-571-87774567-5287 or 5297, or fax to 0086-571-87774553
Email to reagent@bioer.com.cn.
Website: www.bioer.com.cn

Intended Use

This kit is used for qualitative detection of Bovine 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 Bovine.

Principle

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

Ingredients

Product Number	**BSB45S1**	**BSB45M1**
Components	**24T**	**48T**
Sample extraction Buffer	0.5mL/tube×24 Tubes	0.5mL/tube×48 Tubes
Bovine PCR Buffer	1 Tube (548.4 μL)	1 Tube (1096.8 μL)
Taq polymerase	1 Tube (3.6 μL)	1 Tube (7.2 μL)
Negative control	1 Tube (100 μL)	1 Tube (100 μL)
Positive control	1 Tube (100 μL)	1 Tube (100 μL)

Storage and period of validity

1. The Sample extraction Buffer can be transported and stored at room temperature, other components need to be transported or stored at -15℃ ~ -25℃ away from light and avoid repeated freeze-thaw more than 5 times.
2. 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

Thermo Cell thermostatic metal bath, Gene Pure automated nucleic acid extraction system, Line-Gene and Quant-Gene Series Real-time PCR detection system from Bioer or the similar series instrument of other companies.

Sample request

All kinds of raw and cooked meat processed products (including cans and ham sausages, etc.).

Using of the kit

1. Nucleic acid extraction

Select muscle samples from multiple parts, weigh the samples not more than 100mg, cut them into pieces as much as possible, and put them into the Sample extraction Buffer. Swirl well for 5 seconds, warm bath at 80℃ for 10 minutes, mix upside down every 2-3 minutes. Centrifuge at 13000rpm for 5 minutes, absorb 100μL supernatant (avoid oil inhalation) for later use.

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	Bovine PCR Buffer	Taq polymerase
Dosage / test	22.85μL	0.15μL

After mixing PCR reagents above, distribute it into 0.2mL PCR tubes with 23μL per tube. Add 2μL 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℃ 5 min
95℃ 5 sec
62℃ 25 sec } 40 Cycles

Select the FAM channel of instrument for fluorescent signal collection. Instrument set fluorescent signals detecting at 62℃, reagent volume is 25 μL.

Quality control standards

	Result	**Interpretation of Test Results**
Positive Control	Ct value ≤ 28	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 ≤ 30	Positive
30< Ct value < 35	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.
Ct value ≥ 35 or No Ct value	Negative