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Multiple Nucleic Acid Detection Kit for Sheep, Duck, Pig, and Cattle Derived Components (PCR Fluorescence Probe Method)

Product Number: MNK01

Shipping and Storage

Freeze and store below -20℃ in a dark place, with a validity period of 12 months.

Components

Component	48tests
Multiple reaction liquids	1200μL
Positive control (10^6 copies)/μL)	200μL
negative control	1mL

Description

This reagent kit uses probe based quadruple real-time fluorescence quantitative PCR technology to design specific primers and fluorescent probes for nucleic acid sequences of sheep, duck, pig, and cattle derived components. Qualitative and quantitative detection of nucleic acid sequences of sheep, duck, pig, and cattle derived components in meat and meat products is determined through real-time fluorescence quantitative PCR (Taqman probe method) amplification curves.

Applicable instruments:

ABI series (ABI 7300/ABI 7500/Step One, etc.), Roche LightCycle series, Bole CFX 96, Beijing Kunpeng Gene X series, San Shi Biological Q162D, and other real-time fluorescence quantitative PCR instruments.

Protocol

1. Sample preparation (sample preparation area)

   Please refer to the Blood and Tissue DNA Mini Kit or other nucleic acid extraction kits/methods that meet relevant standards to extract nucleic acid from the sample. The extracted nucleic acid should be tested as soon as possible, otherwise it should be stored at -20℃.

2. Preparation of reaction system (sampling area)

   2.1. Remove the multiple reaction tube, positive control tube, and negative control tube. Thaw the reagent completely at room temperature and centrifuge for 10 seconds. Centrifuge the liquid in the tube wall and cover to the bottom of the tube and place it in an ice box for later use.

   2.2. Take the PCR reaction tubes required for the experiment (n+2, i.e. n test samples+positive control+negative control), mix the reaction solution thoroughly by blowing and suction, and then take 24μL of reaction solution into each PCR reaction tube. Then add 1μL of negative control, sample nucleic acid, and positive control in sequence, cover the tube cap, and make a record. The total volume of each reaction is 25μL. Mix thoroughly and centrifuge for 30 seconds before conducting amplification experiments on a real-time fluorescence quantitative PCR instrument.

3. Fluorescent PCR amplification (amplification and product analysis area)

   Pre denaturation at 95℃ for 5 minutes; Denaturation at 95℃ for 10 seconds, annealing at 58℃ for 30 seconds, 40 cycles, and collection of fluorescence signals at 58℃. The fluorescent group is selected as FAM/VIC/ROX/CY5, and the quenching group is selected as None.

4. Result determination

   4.1. The Ct values of the positive control FAM/VIC/ROX/CY5 channels are all less than 30 and show an "S"-shaped amplification curve. The negative control has no Ct value or a Ct value ≥ 40 and no "S" - shaped amplification curve, and

the experimental results are valid; Otherwise, the experiment should be conducted again. If the retest experiment is still ineffective, please contact the technical personnel.

4.2. If the Ct value of the FAM/VIC/ROX/CY5 channel in the sample to be tested is ≤ 36 and an S-shaped amplification curve appears, it is judged as positive. A positive FAM channel indicates the presence of sheep derived components in the sample, a positive VIC channel indicates the presence of duck derived components, a positive ROX channel indicates the presence of pig derived components, and a positive CY5 channel indicates the presence of bovine derived components in the sample.

4.3. The sample test result is 36<Ct<40, indicating suspicion. The sample should be retested. If the Ct value of the retest is less than 40 and there is a clear amplification curve, it is judged as positive, otherwise it is judged as negative.

4.4. If the sample test result shows no Ct value or Ct value ≥ 40 and no "S" amplification curve, it is judged as negative.

Note

1. This reagent kit has high detection sensitivity. In order to prevent pollution, the experiment needs to be strictly partitioned, and it is best to have physical isolation between partitions to avoid cross contamination caused by human factors.
2. Wear work clothes and latex gloves during the experiment, use tools independently in different areas, and change gloves and experimental clothing.
3. The components in the reaction solution are sensitive to light and should be stored away from light. The reagent should be completely thawed before use, but repeated freeze-thaw should be avoided.
4. Please strictly follow the instructions for reagent preparation and sample addition. Instruments such as operating consoles, centrifuges, and pipettes should be regularly disinfected with chlorine containing disinfectants, disinfectant alcohol, nucleic acid contamination removers, or UV lamps.
5. After the reaction is completed, please place the PCR reaction tube in a sealed bag for processing. Do not open the lid to avoid aerosol contamination.
6. Do not mix reagents with different batch numbers and use them within their validity period. Consumables should undergo enzyme free aseptic treatment.
7. A negative test result does not necessarily indicate the presence of non animal derived components and is closely related to the quality of the obtained nucleic acid. Unqualified nucleic acid samples, low nucleic acid load, or unsuccessful nucleic acid extraction (testing) can also result in negative results.
8. Possible reasons for false positives include cross contamination during sample collection, transportation, and nucleic acid extraction.
9. If you have any other questions, please contact the manufacturer's technical personnel in a timely manner. This product is for scientific research purposes only and is not intended for clinical diagnosis or other purposes.