

Real-time PCR Diagnostic Kit for Rapid Identification of Ovine

Product No. MX-T102

Specification 24 Reactions

Principle and Interpretation

This kit adopts real-time fluorescence PCR technology, which is suitable for the in vitro detection of Ovine-derived ingredients in food. Each reaction system contains specific primers and probes for the detection of Ovine-derived ingredients in food, and the qualitative detection of Ovine-derived ingredients is accomplished by collecting the fluorescence signals generated by PCR amplification and the corresponding range of CT values.

Kit Contents

No.	Components	Amount
1	PCR Master mix (Powder)	24 test /Tube
2	RNase-Free ddH ₂ O	1 mL/Tube
3	Positive QC DNA#	1
4	0.2 mL 8-Tube PCR Strips	3

#: Add 24 μ L of RNase-Free ddH₂O into the positive QC DNA tube. Dissolve well and mix well.

Directions

1. Sample Preparation:

Method ①: refer to the preparation described in GB/T 35918-2018;

Method ②: equivalent extraction kits can be used.

2. Prepare the PCR Reaction Mix

① Take out the PCR Master mix (Powder), add 575 μ L RNase-Free ddH₂O to the tube, mix well by pipette or vortex.

Note: If more PCR mix remain, recommended aliquot to the 0.2mL PCR tube for storage for long time use.

② Take 23 μ L PCR mix to 0.2mL PCR tube.

③ Add 2 μ L DNA template from Step 1(Sample Preparation) into PCR tubes, with a total reaction volume of 25 μ L.

④ The corresponding positive control, negative control and sterile deionized water were added to the positive control, negative control and blank control systems respectively.

3. Reaction Condition

Stage	Cycles	Temperature	Time	Step	Fluorescent
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					Signal# Acquisition
Pre-mutability	1	95°C	5min	pre-mutability	No
Real-time PCR	40	95°C	5sec	mutability	No
		60°C	30-60 sec*	metallurgy	Yes

*: When using different types of instruments for time setting, please follow the requirements of the instrument instruction manual for experimental operation, generally set at 30 sec.

#: The Ovine-derived component-specific gene marker was FAM, and the quenching motifs were all TAMRA.

Result Analysis

Blank control: no FAM fluorescence signal was detected and no typical amplification curve appeared.

Negative control: no FAM fluorescence signal was detected and no typical amplification curve was observed.

Positive control (with causative gene): a FAM fluorescent signal was detected, a typical amplification curve was present, and the Ct value was <30.0.

All of the above need to be satisfied at the same time, otherwise this experiment is invalid.

Sample test results:

Ct values ≥ 35 were determined to be negative;

Samples were judged to be positive with a Ct value <30 and a typical amplification curve;

If the Ct value is $30 \leq Ct < 35$ and a typical amplification curve is present, the DNA is reextracted for testing. If the result is still $30 \leq Ct < 35$, the sample is judged as positive; Ct value ≥ 35 , the result is judged as negative.

Limitations of Method

This kit has been validated to provide compositional confirmation for most Ovine breeds, with the possibility of non-detection for a small number of Ovine breeds due to sequence differences; at the same time, the quality of sample collection, handling, transportation and preservation can all have an impact on the results of the assay.

Storage Conditions

Storage at 2-8 °C, or store at -20 °C when not used for a long time.

Shelf Life

24 month.

Announcements

1. Please read the instruction of this kit carefully before the experiment and strictly follow the

operation steps.

2. All components in this kit should be fully melted and mixed before use, and the high speed centrifugation for a short time is necessary.
3. This kit must be stored away from light, and the centrifuge tube and Tips with the DNase and RNase free should be autoclave before used. The whole operation process and the PCR laboratory should comply with the requirements of regulations such as “Administrative Measures for Clinical Gene Amplification Testing Laboratories in Medical Institutions” and “Working Guidelines for Clinical Gene Amplification Testing Laboratories in Medical Institutions” issued by the NHFPC. The waste and amplification products produced during the test should be properly treated to prevent cross-contamination.

For laboratory use in industry or R&D purpose. Not for drug, household or other uses.