Read the instruction leaflet carefully prior to testing. For professional use only.

Cat No.: GLF020

Buffalo Meat Identification Real Time PCR Kit

Buffalo Meat Identification Real Time PCR kit for the qualitative/quantitative detection of nucleic acid from Meat Samples.

INTENDED USE

Buffalo Meat Identification Real Time PCR Kit is designed for the qualitative/quantitative detection of nucleic acid from meat samples. This test system gives detection of Buffalo DNA using real-time PCR.

INTRODUCTION

Accurate identification of animal species, detection of substandard meat and quality control in vegetarian or religiously controlled products is essential to ensure a high level of food safety. Beef inclusion in the meat preparations are serious matters in some religions. Further, the Government of India has allowed export of buffalo tallow only and not the beef tallow. Hence, it is an important task for food control laboratories to be able to carry out species differentiation of raw materials to be used for industrial food preparation and the detection of animal species in food products. Therefore, the need for scientifically based species identification is becoming increasingly important. PCR is an excellent method for the analysis of food and feed samples, enabling rapid and accurate monitoring.

PRINCIPLE

The primer and probe mix targets the specific conserved sequence encoding the *16S rRNA* genes of Buffalo. With the PCR reaction mix provided, the amplification of template can be quantitatively monitored by the increasing fluorescence signal detected by a real-time PCR instrument.

The PCR detection system includes an endogenous internal control primer and probe mix. The result of internal control provides the accuracy of sampling and extraction process, in order to avoid false-negative results.

Buffalo Meat Identification Real Time PCR Kit contains amplification reagents, consisting of the following:

Description	Quantity	Storage
1. Test	10 Vials	RT
2. Positive Control	1 Vial	RT
3. Negative Control	1 Vial	RT
4. Resuspension Buffer	100 µl/vial	RT

INSTRUMENT COMPATIBILITY

Buffalo Meat Identification Real Time PCR Kit is compatible with the following:

Real Time PCR Instruments with channels
FAM
HEX

OTHER MATERIALS APART FROM KIT COMPONENTS, REQUIRED TO PERFORM THE TEST.

- New pair of disposable gloves and facemask
- Personal protective equipment (PPE) kits
- Biohazardous waste container
- DNA extraction kit

STORAGE AND STABILITY

1. Shelf-life of components is 12 months. Manufacture date is indicated on the box.
2. Reagents should be stored in dark at Room Temperature.
3. There constituted liquid reagent should be used up at once.

SPECIMEN REQUIREMENT

1. Sample Type: Meat Samples
2. Sample Collection: Collect in accordance with conventional sample collection methods.
3. Sample Storage and Transportation: Sample to be tested can be processed immediately or stored at –20°C (± 5°C) for 3 months, or –70°C for the long term. Avoid repeated thawing and freezing. Sample should be transported with refrigerant packs in a sealed Styrofoam box or ice chest.

WARNINGS AND PRECAUTIONS

1. This product is to be used only for in vitro diagnostic detection. For use only by laboratory-trained professionals. Please read this manual carefully before use.
2. The contamination of laboratory environment and reagent, or cross-contamination during specimen treatment may lead to a false-positive result.
3. Operation procedure and precautionary warnings of this instrument should be well understood before conducting the test. Quality control should be performed for each test.
4. The decrease of detection effect: A false-negative result may occur if there are any mistakes in the transportation, storage and operation of reagents.
5. Handle all specimens as if infectious, using safe laboratory procedures. All samples should be regarded as potentially infectious materials. Laboratory workers should wear appropriate PPE, including disposable gloves, laboratory coat/gown, etc. Gloves should be changed after handling each sample, to avoid contamination and false results. Laboratory management should be strictly in accordance with the regulations of PCR gene amplification laboratories. Laboratory personnel must be professionally trained, and the experiment process should be strictly divided into sections/organized. All consumables should be properly sterilized and used only once. Instruments and equipment should be assigned to each stage of the experiment and alternative use of the same should be prohibited.
6. Inappropriate sample collection, transfer, storage and operation may lead to inaccurate test results. DNA extraction should be carried out as soon as possible after sample collection to avoid degradation. If it cannot be carried out immediately, it should be stored in accordance with suitable specimen storage procedures. As this test involves the extraction of DNA and PCR amplification, please take care to avoid contamination of the amplification reaction mixture. Also, regular monitoring of laboratory contamination is recommended.
7. When using this kit, please follow the instructions strictly. The collection, storage and transfer of samples, the extraction and detection of DNA, and the interpretation of results must be carried out in strict accordance with the requirements specified in the kit instructions. The processes of sample preparation and addition must be carried out in the biosafety cabinet or other basic protective facilities according to the technical requirements of the regulatory standards.
8. The operation of sample and waste should meet the requirements of relevant laws and regulations. Discard all materials in a safe and acceptable manner, in compliance with all legal requirements. If exposure to skin or mucous membranes occurs, immediately wash the area with large amounts of water. Seek medical advice immediately. Do not use components beyond the expiration date printed on the kit boxes. Do not mix reagents from different lots. Return all components to the appropriate storage condition after preparing the working reagents. Do not interchange vial or bottle caps, as cross-contamination may occur. Reagents supplied are formulated specifically for use with this kit. Make no substitutions in order to ensure optimal performance of the kit. Further dilution of the reagents or alteration of incubation time and temperature may result in erroneous or discordant data.

PREPARATION BEFORE TESTING

Please follow user manual instructions to extract DNA from clinical sample using a DNA extraction kit. Extracted DNA can be used directly for PCR detection. Otherwise, keep DNA sample at –70°C if not in use. Avoid repeated thawing and freezing.

Note: This product does not contain a DNA extraction kit and is compatible with other commercial kits.

DETECTION METHOD

1. TEST

Take out the test vial and add 20µL of the extracted sample to resuspend the mix. Let it sit for 30 seconds, then gently pipette up and down to ensure thorough mixing. Spin the tubes for 20-30 seconds, then transfer the entire volume into a PCR strip or well for testing

2. POSITIVE AND NEGATIVE CONTROL

Add 20 µL of resuspension buffer and gently pipette up and down until the mix dissolved completely and spin the tubes for 30 seconds. Avoid generating air bubbles. Wash the wall of the tube by pipetting thoroughly. Aliquot completely to PCR strip or well.

Note: The reconstituted liquid reagent should be used up at once.

3. SETTING UP PCR

3.1. Place the tubes on the sample holder in the instrument. Set up the test panel according to the positions of positive control, negative control and test samples.

3.2. Select the detection channels as following:
Select FAM (Buffalo) and HEX (Internal control) channels to detect Meat.

3.3. Enter the amplification program commended as below:

	Step	Temperature	Time	Cycle
1	Incubation	50°C	2 minutes	1
2	Initial Denaturation	95°C	10 minutes	1
3	Denaturation	95°C	15 seconds	40
4	Annealing	58°C	30 seconds	
	Extension &Fluorescence measurement	60 °C	60 seconds	
	Cooling	25°C	10seconds	1

Save the file after making the settings and run the reaction. Please set the fluorescence internal control of the instrument to "None". For example, for ABI series instruments set "Passive Reference" to "None".

4. **RESULT INTERPRETATION:** Please refer to the user manual of the instrument for setting the following analysis uses ABI series instruments as an example.
   4.1. After the reaction is completed, the results are automatically saved and the amplification curves of the detected target DNA and the internal control are analyzed separately.
   4.2. According to the analysis, the amplification plot will adjust the Start value End value and Threshold value of the Baseline (users can adjust the values according to the actual situation. Start value can be set within 3~15, and End value can be set within 5~20; users can adjust the amplification curve of negative control to make it linear or below the threshold line). Click "Analyze" to perform the analysis and the parameters should meet the requirements mentioned in "**Section 5. Quality Control**". Lastly, record the qualitative/quantitative results in the Plate window.

QUALITY CONTROL

Buffalo PCR Negative Control:
FAM channel does not show Ct value or Ct>35.

Buffalo PCR Positive Control:
FAM and Internal Control (HEX) channels shows at Ct≤35
The above requirements must be met at the same time in the same experiment otherwise, this experiment is invalid and needs to be repeated.

RESULT INTERPRETATION

Positive Threshold
According to the study of the reference value, the Ct reference value for the target gene detected by this product is 35, and the Ct reference value of internal control is 35.

Result Analysis

Internal control (HEX)	Buffalo (FAM)	Conclusion	Remark
Ct≤35	Has amplification curve; Ct≤35	Has Buffalo meat	Report results to the Sender
Ct≤35	No amplification curve	Negative	Report results to the Sender
Ct>35	-	-	Invalid

1. First, analyze the amplification curve internal control HEX channel. If Ct≤35, it indicates that the detection is valid, and users can continue the subsequent analysis:

a) If a typical S-type amplification curve is detected by the FAM channel, with Ct≤35, it indicates that buffalo meat is present.
b) If FAM channel does not detect a typical S-type amplification curve (no Ct), it indicates that buffalo meat is negative.

2. If the internal control HEX channel failed to detect Ct or Ct>35, it indicates that the concentration of the tested sample is too low or there is an inhibitory reaction from the interfering substance. Users have to repeat the experiment.
3. For negative samples, the internal control should be positive. If the internal control is negative, the test result of the sample is invalid. The cause should be found and eliminated. Users should redo sampling and repeat the experiment (If the result is still invalid, please contact the manufacturer).

LIMITATIONS

1. The test results of this product are for clinical reference only.
2. Analysis of possibility of false-positive and negative results:
   2.1. Improper sample collection, processing and transportation and low sample concentration may cause false-negative results.
   2.2. Variations in the target sequence or sequence changes caused by other reasons may lead to false-negative results.
   2.3. Improper reagent storage can lead to false-negative results.
   2.4. Other unproven interferences or PCR inhibitors may cause false-positive results.
   2.5. Delayed sample processing, may cause false-positive results.
   2.6. This assay should be performed according to Good Laboratory Practice (GLP) regulation. Operators should strictly follow the manufacturer's instructions in performing the test.

PRODUCT PERFORMANCE

Specificity and Sensitivity
The primer and probe provided is designed based on the conserved sequence *16S rRNA* genes of Buffalo and has high detection rate of the target gene fragment.

The specificity of the kit was checked and this product has no cross-reactions among meat samples of Goat, Pig, Dog, Camel, Chicken. The negative and positive rates of detecting commercial reference materials were 100%. The observed values for the Sensitivity of the test kit were 95% and Specificity was 98%.

Results obtained for samples containing potentially interfering substances were compared to results generated to no spiked interference. Each sample was processed in replicates. No interference was observed for samples containing blood.

The negative and positive rates of detecting commercial reference materials were 100%.

Linear Range
The linear range of the Buffalo Meat Identification Real Time PCR Kit for the quantification of Buffalo Meat is 15 - 200000 IU/ml

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09.04.2025

To report any adverse events, write to contact@thegenes4life.com or call us on +91 9778698070

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