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BIOPREMIER

REAL TIME DETECTION KIT Swine

1. PRODUCT DESCRIPTION

Meat detection kits provide a simple, reliable, and rapid procedure for detecting the presence of an animal species. The assay is based on 5' nuclease real time PCR reactions to amplify a unique genomic sequence in the target organism.

2. TECHNOLOGY DESCRIPTION

PCR is a method used to amplify a specific DNA sequence in a reaction containing among other components, a thermostable DNA polymerase, nucleotides, and primers complementary to the target sequence. When this solution is heated, the DNA molecule denatures, separating into two strands. As the solution cools, the primers anneal to the target sequences in the separated DNA strands and the DNA polymerase synthetizes a new strand by extending the primers with nucleotides, creating a copy of the DNA sequence (amplicons). When repeated, this cycle of denaturing, annealing, and extending exponentially increases the number of target amplicons. In Real Time PCR, the signal is measured in each cycle, using in most cases specific fluorescent probes. Fluorescence is measured by a detector and the associated software plots the fluorescence intensity versus number of cycles, allowing the determination of the presence or absence of the target organism.

3. SCOPE

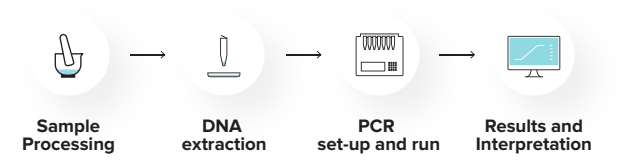
According to the European Commission Directive 2002/86/EC, food ingredients have to be declared. In case of meat and meat products, the animal species has to be disclosed on the label. Moreover, the species authenticity can be highly relevant to consumers for economic, medical, cultural and religious reasons. Fraudulent substitution of cheaper meats in place of more expensive species, the inclusion of meat in non-meat (vegetarian) products and the presence of allergens in food products are clear examples for the importance of this issue (Ballin, 2010; Dooley et al., 2004). Several methods for species detection are based are based on identification of proteins by means of electrophoretic and/or immunological methods. However, these methods are not reliable for application in highly processed and heated products due to the protein deterioration. DNA is more stable than proteins during processing and although it can be fragmented by several processes, modern DNA methodologies like PCR based techniques still allow the identification of DNA from the di□erent species present in a sample (Lockley & Bardsley, 2000). Real time PCR techniques are especially suitable for these products because small fragments of DNA can still be amplified and identified with high sensitivity and specificity (Dooley et al., 2004).

4. INTERNAL CONTROL (IC)

Meat detection kits includes an Internal Control (IC) in the master mix. This control is amplified at the same time as the target DNA sequence, but using a different set of primers and a probe labeled with a different fluorophore. The inclusion of the IC in each reaction allows the evaluation of PCR inhibitors in negative results.

5. APPLICATION

This test allows the detection of swine DNA in food products after DNA extraction. It can also be used with animal feedstuff and other samples in which swine species detection is needed. The specific DNA probe is FAM labeled. The all procedure includes the following main steps:



Note: Sample processing is optional and depends on the sample characteristics.

6. CONTENTS AND STORAGE

The kit contains reagents for 100 assays the sample contains reagents for 10 assays.

VIAL	DESCRIPTION	KIT VOLUME	SAMPLE VOLUME
A ●	Master Mix VIII	2 x 840 µl	168 µl
B ●	10x Swine Assay Mix	210 µl	21 µl
C ●	Positive Control	70 µl	10 µl
D ○	Negative Control	70 µl	10 µl

Store all contents at -20ºC and protect from light as excessive exposure to light may affect the flourescent probes. Minimize freeze-thaw cycles. Reagents stored as recommended may be used until the expiration date indicated in the tube.

7. MATERIAL REQUIRED AND NOT SUPPLIED

- Laminar Air Flow Cabinets/PCR Cabinets
- Disposable powder-free gloves
- Micropipettes and nuclease free filter tips
- Real time PCR instrument
- Tubes/Strips/Multiwell plates and acessories specific for each Instrument
- Freezer, refrigerator
- DNA extraction kit (example: BIOPEXT-0609)

8. PRECAUTIONS AND RECOMMENDATIONS

Molecular Biology procedures, such as DNA extractions and PCR amplification, require qualified staff to prevent the risk of erroneous results, especially due to sample contamination or degradation of the nucleic acids contained in the samples. It is strongly recommended to have dedicated areas, materials and equipments for the DNA extraction, preparation of the PCR and post-PCR procedures. Workflow in the laboratory must proceed in a unidirectional manner, beginning in the Extraction Area and moving to the Amplification and Detection Area.

The user should always pay attention to the following:

- Read all the instructions provided before running the assay.
- Do not mix reagents from different batches
- Wear proper PPE, including disposable gloves and laboratory coats.
- Store and extract positive material separately from all other reagents.
- Thaw all components before starting an assay. When thawed, mix the components and centrifuge briefly.
- Add the positive control after closing all tubes
- Avoid contact of reagents with skin, eyes and mucous membranes. If these solutions come into contact, rinse immediately with water and seek medical advice immediately.

9. PROCEDURE

9.1. Sample preparation

Depending on the specific characteristics of the sample, procedures like homogenization and grinding may be necessary before DNA extraction.

9.2. DNA extraction
Use a kit or protocol suitable for DNA extraction from food products. Follow the manufacturer's or authors instructions.

9.3. PCR preparation

A - PCR mix
Always wear gloves for all PCR procedures.
a)Thaw the kit solutions. Mix thoroughly and centrifuge briefly.
b)To prepare the reactions, add the volumes of the components, as described below:

Note: the volumes indicated below are based on a single standard reaction. Prepare the appropriate mix volume by multiplying the amounts indicated by the number of reactions to be performed (including a positive and a negative control) plus one or two reactions to cover pipetting losses.

DESCRIPTION	VIAL	ASSAY VOLUME
Master Mix VIII	A	16 µl
10x Swine Assay Mix	B	2 µl
Total Volume		18 µl

c) Mix carefully but thoroughly by pipetting up and down (do not vortex). Transfer 18 µl of PCR Mix into each PCR tube.
d) Briefly spin sample tubes before opening to prevent cross contaminations.
e) In separate tubes add:
2 µl of each sample DNA
2 µl from vial D for the negative control
2 µl from vial C for the positive control
f) Close the PCR tubes and briefly spin in a suitable centrifuge.
g) Place the reactions into the Real Time PCR instrument.

B - Program set up
Prepare the Real Time PCR instrument according to the following temperature/time program:

PHASE	STEP	TEMPERATURE	TIME	ACQUISITION
Holding Stage	Step 1	50ºC	2 min.	No
	Step 2	95ºC	5 min.	No
Amplification - 40 cycles	Step 1	95ºC	30 sec.	No
	Step 2	60ºC	30 sec.	Yes
	Step 3	72ºC	30 sec.	No

9.4. Data interpretation
The Probe for swine DNA detection is labeled with **FAM** and must be analyzed in the corresponding fluorescence channel. The Probe to detect the Internal Control (IC) is analyzed in the **ROX** channel. In positive samples, a sigmoidal curve should be observed. This criteria and technician's experience shall prevail over automatic results provided by the software of the instruments.
For each sample, compare the results obtained in each channel and interpret the results as described in the following tables:

a) Controls
To validate the assay, the controls must have the following results:

	Swine DETECTION FAM	IC DETECTION ROX
Negative Control	Negative	Positive
Positive Control	Positive	Negative/Positive

Note that if the controls do not match these results, the experiment must be repeated.

b) Samples
Interpretation of sample results is summarized in the following table:

Swine DETECTION FAM	IC DETECTION ROX	INTERPRETATION
Positive	Positive/Negative	Positive
Negative	Positive	Negative
Negative	Negative	Invalid**

**When both swine and IC detection are Negative, means the presence of PCR inhibitors in the sample. Dilute the sample or perform another DNA extraction.

Note: A prerequisite for unambiguous discrimination of swine DNA and IC DNA in this experiment is a suitable calibration of the channels of the PCR instrument. Please refer to the operation manual of your Real Time PCR intrument.

10. SPECIFICITY
100% Exclusivity, determined using DNA from 23 animal and 3 vegetables species suitable to occur in the same food products.

11. SENSITIVITY
A detection limit of 10 to 100 pg of target DNA can be achieved with REAL TIME DETECTION KIT Swine. About 0,01% of the target species DNA can be detected in food samples if 100 ng of total DNA is used in the reaction.

12. REFERENCES
Ballin NZ (2010). Authentication of meat and meat products. Meat Science, 86:577-587.

COMMISSION DIRECTIVE 2002/86/EC, amending Directive 2001/101/EC as regards the date from which trade in products not in conformity with Directive 2000/13/EC of the European Parliament and of the Council is prohibited. OJ L. 305, 7.11.2002, p.19.

Dooley JJ, Paine KE, Garrett SD, & Brown HM (2004). Detection of meat species using TaqMan real-time PCR assays. Meat Science, 68: 431-438.

Lockley AK, Bardsley RG (2000). DNA - based methods for food authentication. Trends Food Sci. Technol., 11: 67-77.

13. ADDITIONAL INFORMATION
For questions contact: **sales.support@biopremier.com**

BPMR is certified ISO 9001:2015