TABLE OF CONTENTS

1 Product description
2 Technology description
3 Pathogen description
4 Internal Control (IC)
5 Application
6 Contents and storage
7 Material required and not supplied
8 Precautions and recommendations
9 Procedure
10 Specificity
11 Sensitivity
12 References
13 Additional information

BIOPREMIER

REAL TIME DETECTION KIT Horse

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 which is typically amplified in a reaction containing 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, specific fluorescent probes are used to detect the amplified DNA by hybridizing with amplicons. These probes are linked to a fluorophore on one end and a quencher that suppresses fluorescence on the other. If the target sequence is present during the PCR, amplification occurs and the probe is degraded, resulting in fluorescence increase. 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 different 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) DNA in the master mix used in the 5' nuclease assays. 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 second fluorophore. The inclusion of the IC in each reaction avoids false negatives due to the presence of inhibitory substances to PCR and validates any negative result.

5. APPLICATION

This test allows the detection of horse DNA in food products after DNA extraction. It can also be used with animal feedstuff and other samples in which horse species detection is needed. The specific DNA probe is **FAM** labeled.

Sample Processing → **DNA extraction** → **PCR set-up and run** → **Results and Interpretation**

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

6. CONTENTS AND STORAGE

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

VIAL	DESCRIPTION	KIT VOLUME	SAMPLE VOLUME
A	Master Mix IV	2 x 840 µl	168 µl
B	10x Horse 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

- Microcentrifuge
- Heat block
- Micropipettes
- Nuclease free, aerosol resistant pipette tips
- Sterile 1,5 ml microcentrifuge tubes with attached screw-cap
- Real time instruments equipped wih FAM and ROX detection channels
- Real time PCR tubes, strips or plates with optical cap or foil compatible with the PCR thermal cycler in use
- DNA extraction kit (example: BIOPEXT-0609)

8. PRECAUTIONS AND RECOMMENDATIONS

The use of REAL TIME DETECTION KIT Horse involves PCR amplification. The kit provides all reagents required for the PCR. In order to get reliable results the entire assay shall be performed in conditions that avoid nuclease carry over or DNA cross contamination:

- Prepare appropriate aliquots of the solutions, keep them separate from other reagents in the laboratory and use these aliquots rather than directly pipetting from stock solutions.
- Use nuclease-free labware (e.g. pipettes, pipette tips, reaction vials).
- Wear gloves when performing the assay.
- To avoid cross-contamination of samples and reagents, use fresh aerosol-preventive pipette tips.
- Add the positive control after closing all tubes
- If possible, physically separate the workplaces for DNA preparation.

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 and briefly spin vials before opening to ensure the complete recovery of volumes. Mix carefully but thoroughly by pipetting up and down.
b) To prepare 20 µl standard reactions, prepare the PCR Mix by adding the following volumes of kit components:

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 IV	A	16 µl
10x Horse 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 contamintions.
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
Probe for horse DNA detection is labeled with **FAM** and must be analyzed in the corresponding fluorescence channel. Probe to detect the specific amplification of Internal Control (IC) is analyzed in the **ROX** channel. 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:

	Horse DETECTION FAM	IC DETECTION ROX
Negative Control	Ct = NA	Positive
Positive Control	Positive	Not significant

NA: Not Applicable. It corresponds to signal that do not cross the threshold.

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:

Horse DETECTION FAM	IC DETECTION ROX	INTERPRETATION
Positive	Not significant	Positive
Ct = NA	Positive	Negative
Ct = NA	Ct = NA	Inhibition**

**When both Horse and IC detection are NA, the sample must be tested again after 1/10 dilution.

Note: A prerequisite for unambiguous discrimination of horse 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 cycler.

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 Horse. About 0,01% of the target species DNA can be detected in food samples if 100 ng of DNA is used in the reaction.

12. REFERENCES
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