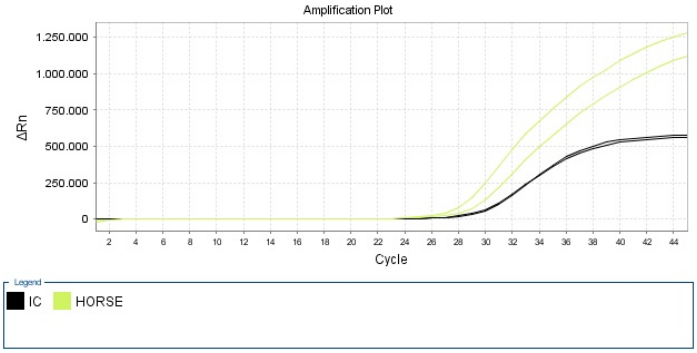
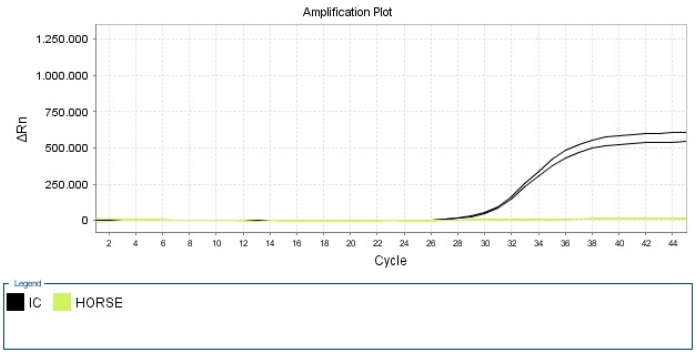
Positive Control –



Negative Control –



12. SPECIFICITY/INCLUSIVITY

a) 100 % Exclusivity, determined using non-taget DNA species from animal species suitable to occur in the same food products (Tables 1, 2).
b) 100 % Inclusivity, determined in food samples obtained from different commercial sources and from External Quality Control (Table 3).

13. SENSITIVITY

The limit of detection (LoD) for SUPREME REAL TIME DETECTION KIT Horse is 10 pg of target DNA.
The method's detection limit can detect 0.02% of equine (*Equus ferus caballus*) DNA in 50 ng of total DNA.
No false negatives were obtained, either in the defined PCR conditions or in the deviations used for robustness, increasing the stringency.

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BPMR is certified ISO 9001:2015

BIOPREMIER

SUPREME
REAL TIME DETECTION KIT

HORSE

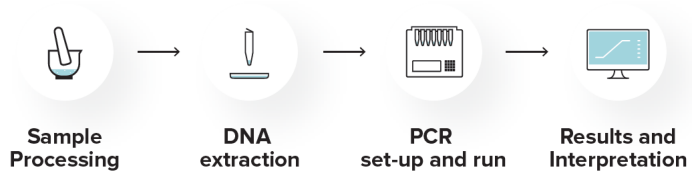
Ref: BIOPSFS-0008

1. BACKGROUND INFORMATION

According to the European Commission Directive 2002/86/EC, food ingredients have to be declared. Species authenticity is highly relevant to consumers for economic, medical, cultural, and religious reasons. Examples like fraudulent substitution of expensive species with cheaper meats, inclusion of meat in vegetarian products, and allergens in food products highlight the importance of this issue. Additionally, meat species must be disclosed on the label with both commercial and scientific denominations to verify the meat's origin and traceability and to analyze the quality control of handling and cleaning processes of production lines. Methods for species detection based on the identification of proteins are not reliable for application in highly processed and heated products due to protein deterioration. DNA is more stable than proteins during processing. Traditional protein-based detection methods are unreliable for highly processed and heated products, unlike DNA-based methods. DNA is more stable, and modern PCR techniques enable the identification of fragmented DNA from the species present in a sample, ensuring accurate and highly sensitive detection with a lower detection limit detection. Real time PCR techniques are suitable for amplifying small fragments of DNA high sensitivity and specificity.

2. INTENDED USE

The SUPREME REAL TIME DETECTION KIT Horse enables a qualitative detection of equine (*Equus ferus caballus*) DNA in food samples by Real Time PCR, after a sample processing step. This DNA is present in the mitochondrial genome and it may be found in processed food and food industry. The kit includes an Internal Control (IC). The IC is used to evaluate PCR inhibitors in the sample or to evaluate problems that occurred during PCR preparation/amplification. The kit includes a Master mix for the target and IC, primers and TaqMan® probes, labelled with non-fluorescent quenchers. The kit contains Uracil-DNA Glycosylase (UDG), preventing DNA contamination with PCR products. The kit includes a positive control, which allows an evaluation of the primers and probes used to detect the targets, and a negative control (nuclease-free water) to confirm the integrity of the kit reagents.
The kit was validated in the instruments ABI PRISM® 7500 Fast. The kit is compatible with all thermocyclers working in **FAM** and **ROX** channels. The detection kit must not be used for diagnostic procedures. For Food use only.
The procedure includes the following main steps:



3. TRADEMARKS AND DISCLAIMER

Registered names, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law. BPMR kit handbooks and user manuals can be requested from BPMR or your local distributor.

4. LIMITED LICENSE AGREEMENT

Use of this product signifies the agreement of the following terms: The kit must be used solely in accordance with the respective Instructions for Use. BPMR grants no license under any of its intellectual property to use or incorporate the enclosed components of this Kit with any components not included within this kit except as described in the Instructions for Use. This kit and its components are licensed for one-time use and may not be reused, refurbished, or resold.

5. QUALITY CONTROL

In accordance with BPMR's ISO 9001, each lot of the kit is tested against predetermined specifications to ensure consistent product quality.

6. WARNINGS AND PRECAUTIONS

Molecular Biology procedures, such as RNA 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 equipment for the RNA 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.

7. CONTENTS AND STORAGE CONDITIONS

The kit should be stored between -20°C and 5°C and protected from exposure to light. Repeated thawing and freezing (>5) should be avoided, as this may reduce the sensitivity. Considerate to freeze in aliquots to maintain the performance of the assay.
The kit contains reagents for 100 reactions.

CONTENTS	UNITS	COMPOSITION
Master Mix (blue cap)	2 tube (2 x 683µl)	Buffer, dNTPs, Primers and Probes
Enzymes (yellow cap)	1 tube (1 x 220µl)	DNA polymerase, UDG and storage buffer
Negative Control (clear cap)	1 tube (1 x 130µl)	Nuclease-free water
Positive Control (red cap)	1 tube (1 x 130µl)	Target DNA

8. MATERIAL REQUIRED AND NOT SUPPLIED

- Microcentrifuge
- 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 accessories specific for each Instrument
- DNA extraction kit (example: BIOPEXT-0609)

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, such as BIOPREMIER DNA Extraction Kit from Food (ref: BIOPEXT-0609) or similiar. Follow the manufacturer's or authors instructions.

9.3 PCR preparation

A – PCR mix

Always wear gloves for all PCR procedures.

1. Thaw the kit solutions. Mix thoroughly (do not vortex the tube Enzymes) and centrifuge briefly
2. Prepare the reactions, as described below:

CONTENTS	Nº OF SAMPLES	
qPCR reaction	**1**	**10 (10 + 1)**
Master Mix	13 µl	143 µl
Enzymes	2 µl	22 µl
Total Volume	**15 µl**	**165 µl**

Note: Prepare the PCR reaction for each sample, or in alternative, prepare a Master Mix for the total number of reactions plus 10% (e.g. for 10 samples, prepare a volume for 11). In this case, prepare the Mix in a 1,5mL sterile, nuclease-free tube. Include 2 PCR reactions for the Positive and Negative controls.

3. Mix the prepared Mix by inverting the tube and centrifuge briefly
4. Dispense 15 µL aliquots of prepared Mix into the plate wells or PCR tube
5. For the negative control, pipette 5 µL of Negative Control tube (Clear Cap); Pipette 5 µL of DNA sample per well; and for the positive control, pipette 5 µL of Positive Control tube (Red Cap). Each PCR tube / well should have a final PCR volume of 20 µL
6. Centrifuge briefly the plate wells or PCR tubes
7. 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	TEMPERATURE	CYCLES	TIME	ACQUISITION
Incubation	37 ºC	1	15 min	No
UDG Inactivation	95 ºC	1	5 min	No
DNA Amplification	94 ºC	45	15 s	No
	60 ºC		30 s	Collect Data
	72 ºC		15 s	No

Sample Volume: 20 µl
Passive reference dye: None
Detection format: Hydrolysis Probe

TARGET	CHANNELS
Detection of Horse	FAM | Excitation at 465 nm, Emission at 510 nm
Detection of Internal Control (IC)	ROX | Excitation at 533 nm, Emission at 610 nm

14. DATA ANALYSIS

For analysis of PCR results, select fluorescence display options. Samples with positive Ct-values are considered positive.
Important: Please, also check amplification curves, not only Ct values. Samples should be inspected both in logarithmic and linear scale view and compared with the negative control. Adjust the Threshold, if necessary. Sample results should be assessed after the positive and negative controls have been examined and determined to be valid. If the results of controls are not valid, the sample results cannot be interpreted.
Interpretation of PCR-data:

a) Controls

To validate the assay, the controls must have the following results:

	Horse DETECTION FAM	IC DETECTION ROX
Negative Control	Negative	Positive
Positive Control	Positive	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:

Horse DETECTION FAM	IC DETECTION ROX	INTERPRETATION
Positive	Positive/Negative**	DNA detected, sample Positive for Horse
Negative	Positive	No DNA detected, sample Negative for Horse
Negative	Negative	Invalid Result*

*When both Horse and IC detection are Negative, means the presence of PCR inhibitors in the sample. Dilute the sample or perform another DNA extraction.
** High DNA concentration of the target in the sample can lead to a reduced or absent fluorescence signal of the IC.

Table 1: Food samples tested for validation of the SUPREME REAL TIME DETECTION KIT Horse

Food samples
Pork lasagna
Lamb
Shredded duck
Wild boar
Corn
Potato
Goose foie gras
Duck foie gras
Soy
Rabbit
Pork sausages
Chicken steak
Vegetarian meatballs
Mortadella
Chicken hamburgers
Beef hamburger
Horse meat

Table 2: List of species used for exclusivity of the SUPREME REAL TIME DETECTION KIT Horse

Species
Sus scrofa domesticus
Ovis aries
Sus scrofa
Zea mays
Solanum tuberosum
Anser anser domesticus
Anas platyrhynchos
Glycine max
Oryctolagus cuniculus
Gallus gallus domesticus
Bos taurus

Table 3: Analytical Sensivity of the SUPREME REAL TIME DETECTION KIT Horse

Food samples	Horse %	LoD (pg)	[horse] (pg)	#total	#positive
Horse meat	100%	10	10	11	11
Meatballs + Horse meat	10%	10	1	10	10
Pork sausages + Horse meat	10%	10	1	10	10
Beef hamburger + Horse meat	10%	10	1	10	10
Analytical Sensitivity					**100%**