For food testing purposes. FOR *IN VITRO* USE ONLY.

foodproof® SL Horse Species Detection Kit

- 5'Nuclease -

Version 1, October 2014

PCR kit for the qualitative detection of Horse species DNA using real-time PCR instruments.

Order No. Z 730 04

Kit for 50 reactions for a maximum of 48 samples

Store the kit at -15 to -25 °C

Table of contents

1. INTRODUCTION

To assure a high level of food and feed safety, accurate animal species identification and the detection of adulterants are two of the greatest challenges facing food and feed products companies today. Therefore, the need for scientifically valid species identity methods is increasingly important. Although a number of traditional morphological, microscopic and chemical methods have commonly been used for species identity testing, technologies using DNA offer reliable alternative methods that can provide increased precision in differentiating closely related species, as well as identifying intentional and accidental adulterants and contaminants.

The Animal Species detection kits are designed for use by food and feed producers, dairies, marketers of these products, as well as regulators and auditors of final food and feed quality and safety. It is also intended to be used to verify that ruminant feed and feed supplements are properly labeled and do not contain ruminant materials.

2. INTENDED USE

foodproof SL **Horse Species Detection Kit** is designed to detect the specific gene for horse species in feed, food, cosmetics and pharmaceuticals and *etc*. This kit provides real-time PCR Master Mix with enzyme components and the specific primer/probe set for rapid testing by real-time PCR assay, as well as the Internal Control (IC) system for reliable results.

3. PRINCIPLE OF PCR DETECTION

Horse Detection by real time PCR assay is a qualitative Duplex real-time PCR test, for the detection of horse specific gene and the Internal Control (IC) using specific primers and probes labeled with the fluorescent dyes. The target sequences are detected through the **FAM and HEX (VIC)** channel respectively.

The primer and probe mixture provided exploits the so-called TaqMan® principle. During PCR amplification, forward and reverse primers hybridize to the target DNA. A fluorogenic probe is included in the same reaction mixture which consists of an oligonucleotide labeled with a 5'-reporter dye and a downstream 3'-quencher. During PCR amplification, the probe is cleaved and the reporter dye and quencher are separated. The resulting increase in fluorescence can be detected on a range of real-time PCR platforms. The monitoring of the fluorescence intensities during the real-time PCR allows the detection of accumulating product without re-opening the reaction tubes after the PCR run such as electrophoresis.

The kit minimizes contamination risk and contains all reagents needed for detection (except for H_2O PCR-grade).

- **Internal Amplification Control**

This kit contains the Internal Control (IC) as PCR inhibition Control. The IC allows the user to determine and control possible PCR inhibition. The IC reagents are included in the primer/probe Mixture and the IC is co-amplified with target DNA from specimen. The results can be visualized in the **HEX (VIC) channel**.

4. CONTENTS

This kit is intended for 50 reactions, including controls.

Table 1: Kit Contents

Reagent	Cap label	Volume	Description
2x real-time PCR MasterMix	2xM	500 µl	Buffer containing dNTPs, $MgCl_2$ and Taq DNA polymerase
Primer / Probe Mixture	P	200 µl	Primer/ probe mixture - Horse specific primer and probe - IC-specific primer and probe - DNA for IC
Control DNA	C	50 µl	Positive control DNA

5. ADDITIONALLY REQUIRED MATERIALS, REAGENTS AND DEVICES

- Disposable powder-free gloves and laboratory coat
- Pipettes (capacity 0.5~10 µl, 2~20 µl, 20~200 µl, 200~1,000 µl)
- Sterile pipette filter tips with aerosol barriers
- Ice maker
- Vortex mixer
- Clean bench or PCR box
- Desktop centrifuge with rotor for 2 ml reaction tubes
- Real-time thermo cycler with FAM and HEX (VIC) detection channels
- Disposable polypropylene micro tubes for PCR
- H_2O PCR-grade
- For DNA Extraction: **food**proof® Sample Preparation Kit III (available from BIOTECON Diagnostics; see Ordering Information for details)

6. GENERAL PRECAUTIONS

- Store extracted positive material (samples, controls and other amplicons) away from all other reagents and add it to the reaction mix in a separate area.
- Thaw all components thoroughly on ice before starting experiment.
- When thawed, mix the components and centrifuge briefly.
- Do not pipette by mouth.
- Do not eat, drink, smoke, apply cosmetics or handle contact lenses in laboratory work areas.
- Do not use a kit after its expiration date.
- Material Safety Data Sheets (MSDS) can be requested, please refer to www.bc-diagnostics.com
- Use disposable gloves, laboratory coats and eye protection while samples and reagents handling. Thoroughly wash hands afterwards.
- Dispose of all samples and unused reagents in compliance with local regulations.
- Specimens should be considered potentially infectious and handled in biological cabinet in accordance with Biosafety Level 2 or other appropriate biosafety practices.
- Clean and disinfect all sample or reagent spills using a disinfectant such as 0.5% sodium hypochlorite, or other suitable disinfectant.
- Avoid contact of specimens and reagents with the skin, eyes and mucosa. If skin, eyes and mucosa contact immediately flush with water, seek medical attention.
- Use of this product should be limited to personnel trained in the techniques of DNA amplification.
- To avoid carry-over contamination with PCR product or control DNA, please note the following points:

1. Please be careful not to contaminate the Primer/Probe Mixture and 2x real-time PCR MasterMix with PCR products or Control DNA through pipetting. To prevent contamination, use of filter tips is recommended.
2. Open and close all sample tube carefully. Avoid splashing or spraying PCR samples.
3. It is important to have designated areas of the lab where PCR reactions are set up, preferentially separated in space from the areas where PCR reactions are analyzed by gel electrophoresis.
4. The laboratory process must be one directional, it should begin in the Extraction Area move to the Amplification and Detection Area. Do not return samples, equipment and reagents in the area where you performed previous step.

7. SAMPLING AND HANDLING

7.1 **Sample Collection**

Various food source sample, environmental sample, clinical material and cultured bacteria are routinely examined.

7.2 **Sample Storage**

The sensitivity of the assay can be reduced if you freeze the samples as a matter of routine or store them for a longer time. Please avoid repeat freezing and thawing of samples which may lead to the degradation of DNA and decreased sensitivity.

7.3 **Nucleic Acid Extraction**

Various manufacturers offer DNA isolation kits. Carry out the DNA isolation according to the manufacturer's instructions. For more information please refer to www.bc-diagnostics.com

8. PROTOCOL

8.1 **DNA Isolation**

BIOTECON Diagnostics provides sample preparation kits suitable for all kind of foods and raw materials (see 5. “Additional Required Materials, Reagents and Devices”)

8.2 **Preparing the PCR**

To prevent the risk of contamination with foreign DNA, we recommend that all experiment steps be performed in a PCR clean room or separated environment area. Filter tips are recommended for each step.

8.2.1 **Thawing the kit components on ice.** Using ice or lab top cooler is recommended during experiment for maintaining the enzyme activity.

8.2.2 Total reaction volume is 20 µl the volume of DNA sample is 6 µl. **Prepare a reaction mixture according to Table 2.**

Table 2: PCR reaction mixture

Composition	Volume
Primer / Probe Mixture	4 µl
2x real time PCR MasterMix	10 µl
Total	14 µl

Add 6 µl of extracted DNA sample into the tube.

8.2.3. Carry out the control amplification reactions.

CONTROL +

- Positive control amplification: Add 6 µl of Control DNA instead of sample DNA.

CONTROL -

- Negative control amplification : Add 6 µl of H_2O PCR-grade instead of sample DNA

8.2.4. Mix the reagents in the PCR reaction tubes by tapping minimum of 5 times. Briefly centrifuge the tubes to remove air bubble and drops from the inside of the cap.

8.3 **Amplification**

- Program your real-time PCR instrument according to manufacturer’s manual.
- Create a temperature time profile on your instrument as follows in Table 3.

Table 3: Temperature Time Profile

Temperature	Time	Cycle
95 ℃	10 min	1
95 ℃	15 sec	35
61 ℃ *	40 sec	

* Detect the fluorescence at this step.

9. DATA ANALYSIS

The fluorescence curves are analyzed in FAM and HEX (VIC) fluorescence detection channels (see Table 4).
You can predict the presence or absence of target gene in your samples by analyzing the real-time PCR result.

Table 4: Specific Detection on Fluorescence Channel

Target Gene	Fluorophore
Horse specific gene	FAM
IC	HEX (VIC)

9.1 **Interpretation of Results**

- The signal is considered to be positive, if the corresponding fluorescence accumulation curve crosses threshold line. Results are accepted as relevant if both positive and negative controls of amplification are passed.
- **IC**: When amplifying a target sample with a high copy number, the IC may not produce an amplification plot. This does not invalidate the test and should be interpreted as a positive experimental result.

Table 5: Interpretation of Results

	Positive Control	Negative Control	FAM Horse specific gene	HEX (VIC) IC	Interpretation
Case 1	+	-	+	+	The horse specific gene is detected in a sample.
Case 2	+	-	+	-*	The horse specific gene is detected in a sample.
Case 3	+	-	-	+	The horse specific gene is not detected in a sample.
Case 4	+	-	-	-	invalid result/retest
Case 5	+	+	+/-	+/-	
Case 6	-	+	+/-	+/-	
Case 7	-	-	+/-	+/-	

* Detection of the Internal Amplification Control in the respective channel is not required for positive result.
A high copy number of target gene can lead to reduced or absent Internal Amplification Control signal.

10. TROUBLESHOOTING

Situation	Possible cause	Recommendation
Negative control samples are positive.	Carry-over contamination	▪ Exchange all critical solutions. ▪ Repeat the analysis of all tests with fresh aliquots of all reagents. ▪ Take measures to detect and eliminate the source of contamination.
No signal is detected for positive controls of amplification.	Incorrect programming of the real-time PCR instrument.	The PCR should be repeated after check for programming of instruments, storage conditions and the expiration date.
	The kit reagents have expired.	
	The storage conditions for kit components have not complied with manufacturer instruction.	
	Incorrect PCR reaction ▪ Pipetting errors ▪ Omitted reagents	The PCR should be repeated after check for correct pipetting scheme and reaction setup.
No signal is detected for IC on HEX (VIC) channel and horse specific gene on FAM channel.	PCR inhibitors are present at a high concentration.	DNA extraction should be repeated.

If you have any further questions or encounter problems, please contact us.

Email: bcd@bc-diagnostics.com
Tel: +49-(0)331 2300-200

11. STABILITY AND STORAGE

Store the kit at –15 to –25 °C through the expiration date printed on the label.

12. SPECIFICATIONS

- **Sensitivity**

  0,1 GE limit of detection (LOD)

- **Specificity**

  100% exclusivity for about 100 non-specific species DNAs

13. QUALITY CONTROL

In compliance with Federal State Institution of Science “Central Research Institute of Epidemiology” ISO 13485 – certified Quality Management System, each lot of **food**proof® SL **Horse Species Detection Kit** has been tested against predetermined specifications to ensure consistent product quality.

14. ORDERING INFORMATION

Product	Order No.	Unit
foodproof® SL Horse Species **Detection Kit**	Z 730 04	50 rxn
foodproof® **Sample Preparation Kit III ***	S 400 06.1	50 rxn

* NOTE: use the **food**proof® Sample Preparation Kit III optimized protocol for Animal Identification, please refer to:

http://www.bc-diagnostics.com/

15. SUPPLEMENTARY INFORMATION

15.1 **Trademarks**

foodproof® is a trademark of BIOTECON Diagnostics GmbH.

Other brand or product names are trademarks of their respective holders.

15.2 **Change Index**

Version 1:

First version of the package insert.

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