

RapidFinder™ Equine ID Kit

SKU: A15570

For testing of Food and Environmental samples only.

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1. Kit description

Identification of meat species presence in food samples is an essential step in order to verify the origin and traceability of the used raw materials, as well as a necessary quality control for handling and cleaning processes of production lines.

RapidFinder™ Equine ID Kit allows determining the presence of DNA of horse (*Equus caballus*) in any food.

Equine DNA detection is done by real time PCR using two TaqMan®-MGB probes. One of them, labelled with FAM™ dye, specifically detects one mitochondrial DNA sequence of *Equus caballus*. The second probe is labelled with VIC® and detects an Internal Positive Control, which is used to rule out inhibitors in the sample and check the correct functioning of the assay.

The detection limit has been calculated using standard samples consisting of mixtures of raw beef meat and other species. **RapidFinder™ Equine ID Kit** can detect blends containing a percentage below 0.1% (w/w) of beef meat. The limit of detection in processed samples varies depending on the composition and food processing.

To ensure the representativeness of the results, we recommend the use of a DNA extraction method that allows you to process a large amount of sample (10-20 g). If you do not have a procedure with these features, we recommend the use of **GMO Extraction Kit** (Part No: 4466336).

2. Specificity

The specificity of the kit was tested through comparison with the NCBI sequence database and was also experimentally tested with success on a collection of reference DNAs. See the results in the table below:

Meat Species	Results
Sheep	ND
Goat	ND
Horse	D
Beef	ND
Pork	ND

Meat Species	Results
Deer	ND
Chicken	ND
Turkey	ND
Duck	ND
Ostrich	ND
Goose	ND
Human	ND

D: Detected

ND: Not Detected

3. Kit Contents and storage

The Kit contains the necessary reagents to perform 48 reactions:

Reagents	Identification	Amount	Storage
Equine Master Mix	Red pad	378 µl	-20°C
General Master Mix	White pad	630 µl	4°C
Positive Control	Orange cap	60 µl	-20°C

4. Equipment requirements

In the following table the equipment requirements for using **RapidFinder™ Equine ID Kit** are shown:

EQUIPMENT	
1	Real-time PCR Thermal Cycler with channels for detection of FAM™ (520 nm) and VIC® (550 nm)
2	Micropipettes (10 µl, 20 µl and 200 µl)
3	Table top centrifuge with adaptors for 96 well PCR plates and/or 0,2 ml tubes
4	Vortex

5. Consumables

Needed consumables are shown in the following table:

MATERIALS	
1	Optical 96-well reaction plates or 0.2 ml optical tubes
2	Optical adhesive film for 96 well plates or optical adhesive covers for 0.2 ml tubes
3	Disposable micropipette filter tips
4	1.5 ml sterile tubes
5	Powder-free latex gloves

6. Amplification reactions procedure

RapidFinder™ Equine ID Kit is designed to determine, in a single PCR reaction, the presence or absence of bovine DNA and the internal positive control.

We recommend using, the positive control included in this kit for each run. This positive control contains 0.1% of equine DNA.

To estimate the amount of necessary reagents, we recommend make calculations taking into account the number of samples and controls to be simultaneously analysed, and then considering one more reaction, or increase a 10% the volume of each reagent.

The recommended protocol for preparation of amplification reactions is showed below:

1. Thaw the two Master Mix and the Positive Control vial
2. Vortex each reagent and keep cold.
3. In a 1.5 ml tube, add 7.5 µl of Beef Master Mix and 12.5 µl of General Master Mix for each reaction included in the same PCR run. Vortex and pipette 20 µl per well or tube of 0.2 ml.
4. Add 5 µl of each DNA sample at 10 ng/µl, into the appropriate wells. We recommend making each sample analysis in duplicate.
5. Add 5 µl of Positive Control and Negative Controls* into the appropriate wells.
6. Cover the well plate with optical film or the tubes with optical cover and spin in the centrifuge.

** We strongly recommend using an **extraction negative control** for each run of extractions carried out. This control consists in one tube to which no sample is added and which is summited to the same extraction process as the other samples. Likewise, we recommended using a **PCR negative control** for each PCR run; this tube contains no DNA but all PCR reagents.*

7. PCR Amplification program

Amplification reactions must be submitted to the following PCR program:

Temperature	Time	Cicles
95°C	10 minutes	1
95°C	15 seconds	36
60°C	1 minute	

Note: This program has been validated on a StepOne Real-Time PCR System from Applied Biosystems. If you use another brand or model of thermal cycler, you may need the amplification program to be adjusted. Please contact our service department for advice.

8. Analysis of results

Before analysing the samples results, you should check that the results obtained in the controls, is as expected:

- **Positive Control:** The result must always be positive in all amplification reactions, both in the FAM™ channel as VIC®.
- **Negative controls:** Amplification must be only detected in the VIC® channel. In this channel an internal positive control (IPC) is detected, which determines the absence of inhibition in the sample.

IPC

It must be checked that the IPC (VIC®) is positive in all samples, with a Ct similar to the Positive Control. A negative result in the IPC indicates the presence of inhibitors in the sample. It should be noted that IPC result may be negative in samples where a lot of bovine DNA (FAM™) is detected, because the PCR reagents are exhausted before amplification of the IPC begins.

Bovine

Amplification in the FAM™ channel indicates presence of equine DNA in the sample.

It is necessary to check if sample Ct is less than the Ct_{cut-off} in order to determine if one reaction of amplification is positive. Any reaction of amplification with Ct upper than Ct_{cut-off} may be considered as negative. The Ct_{cut-off} is equal than the positive control Ct (0.1%) plus 3.32.

$$Ct_{cut-off} = 3.32 + Ct_{Positive\ Control}$$

Note: Any sample with a Ct equal than Ct_{cut-off} contains approximately 0.01% of equine DNA.

In samples where no amplification in the FAM™ channel is seen, we can conclude that no bovine DNA is detected or that its amount in the sample is below than the detection limit.

The following table shows graphically the results that may be obtained from one sample analysis, as well as the interpretation that should be done from the obtained result:

Master Mix Beef		INTERPRETATION
Beef	IPC	
-	+	No equine DNA is detected
+	+	Equine DNA is detected
-	-	PCR inhibitors presence in the sample*
+	-	Sample with big amount of equine DNA

** If presence of inhibitors in the sample is detected, we recommend checking whether there has been an excess of DNA in the reaction (the recommended maximum is 250 ng). If the amount of DNA is right, we recommend repeating DNA extraction. If the problem persists, please contact our technical department.*

The following table shows graphically the results that may be obtained from the analysis of different assay controls, as well as the interpretation that should be done from the obtained result:

Controls	Master Mix Beef		INTERPRETATION
	Beef	IPC	
Positive Control	+	+	Expected result
	-	-	PCR Amplification Failure ¹
Extraction Negative Control	-	+	Expected result
	+	+	Contamination in the bovine DNA extraction procedure ²
PCR Negative Control	-	+	Expected result
	+	+	PCR contamination with bovine DNA ³

Recomendations:

¹ **PCR Amplification Failure:** Check amplification program and configuration of fluorescence capture. Amplification failure may be due to a setup technical problem.

² **Contamination in the equine DNA extraction procedure:** Contamination may be due to some error made in the process of sample handling, reagents contamination, or environmental contamination. Check DNA extraction protocol, wipe the laboratory where DNA extraction process was performed and take care to avoid any contamination during sample homogenization. If necessary, use new aliquots of the reagents used in DNA extraction.

³ **PCR contaminations with equine DNA:** Contamination of PCR reactions may be due to an error made in the process of sample handling, contamination of the reagents or environmental contamination. Thoroughly clean the laboratory where the PCR process was performed, as well as equipment. If necessary, use new aliquots of the reagents used in the PCR. Prepare the PCR reaction containing the Positive Control last to avoid cross contamination.

9. Quantitative analysis

In case of positive results, it is possible to determine the percentage of bovine DNA versus total animal DNA present in one sample. We can use this kit in combination with **RapidFinder™ Quant Equine Set (SKU A15579)**

Quantification assay is based in two absolute quantifications. One of these quantifications is performed making five serial dilutions from equine master mix (included in the RapidFinder™ Quant Equine Set) and doing PCR reactions using RapidFinder™ Equine ID Kit (mixing Equine Master Mix with General Master Mix). This quantitative assay determines the total amount of equine present in one sample. The second quantification is obtained doing PCR reactions using RapidFinder™ Quant Equine (mixing Animal Master Mix with General Master Mix). This quantification determines the total amount of animal present in one sample.

To design the animal TaqMan® probe we selected a highly conserved mitochondrial genomic region.

For more information about quantitative analysis see the **RapidFinder™ Quant Multi-Meat Set** user manual (www.imegen.es), or contact our local distributor

10. Quality control

All products manufactured and marketed by the Instituto de Medicina Genómica are submitted to a rigorous quality control process. This kit has passed all the internal validation tests, thus guaranteeing the reliability and reproducibility of each assay.

The Certificate of Analysis corresponding to your kit can be consulted by entering the batch number in the Analytical Kits section on the web page www.imegen.es.

11. Customer support

For the latest services and support information for all locations, go to:
www.lifetechnologies.com/support

Food Safety support

Website: <http://www.lifetechnologies.com/foodsafety>

Support email: foodsafety@lifetech.com

Phone number in North America: 1-800-500-6855

Phone number outside of North America: Visit www.lifetechnologies.com/support, select the link for phone support, and select the appropriate country from the dropdown menu.

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