RapidFinder™ Ruminant ID Kit

SKU A24396

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™ Ruminant ID Kit allows determining the presence of DNA of ruminant (*Bos taurus, Ovis aries, Capra hircus, Cervus elaphus and Capreolus capreolus*) in any food.

Ruminant DNA detection is done by real time PCR using two TaqMan®-MGB probes. One of them, labelled with FAM™ dye, specifically detects one nuclear DNA sequence of ruminant. 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 upon standard samples consisting of mixtures of raw ruminant meat and other species. **RapidFinder™ Ruminant ID Kit** can detect blends containing a percentage below 0.1% (w/w) of ruminant 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. It showed no similarity with other non-ruminant species. The specificity was also experimentally tested with success on a collection of reference DNAs. See the results in the table below:

Meat Species	Results
Sheep	D
Goat	D
Horse	ND
Beef	D

Meat Species	Results
Deer	D
Pork	ND
Donkey	ND
Rabbit	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 contents the necessary reagents to perform 48 reactions:

Reagents	Identification	Amount	Storage
Ruminant Master Mix	Red pad	403 µl	-20ºC
General Master Mix	White pad	605 µl	4ºC
Positive Control	Red cap	60 µl	-20ºC

4. Equipment requirements

In the following table the equipment requirements for using **RapidFinder™ Ruminant 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™ Ruminant ID Kit is designed to determine, in a single PCR reaction, the presence or absence of ruminant 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 ruminant 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 8 µl of Ruminant Master Mix and 12 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 summited to the following PCR program:

Temperature	Time	Cicles
95ºC	10 minutes	1
95ºC	15 seconds	36
60ºC	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 ruminant DNA (FAM™) is detected, because the PCR reagents are exhausted before amplification of the IPC begins.

Ruminant

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

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

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

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

In samples where no amplification in the FAM™ channel is seen, we can conclude that no ruminant 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 Ruminant		INTERPRETATION
Ruminant	IPC	
-	+	No ruminant DNA is detected
+	+	Ruminant DNA is detected
-	-	PCR inhibitors presence in the sample*
+	-	Sample with big amount of ruminant 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 Ruminant		INTERPRETATION
	Ruminant	IPC	
Positive Control	+	+	Expected result
	-	-	PCR Amplification Failure[1]
Extraction Negative Control	-	+	Expected result
	+	+	Contamination in the ruminant DNA extraction procedure[2]
PCR Negative Control	-	+	Expected result
	+	+	PCR contamination with ruminant 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 ruminant 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 ruminant 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. Ruminant DNA detection in Processed Animal Protein (PAP)

This kit can also detect the presence of DNA in Processed Ruminant Animal Protein (PAP).

To perform this assay please, follow the instruction on the protocol of the European Union Reference Laboratory

http://eurl.craw.eu/img/page/sops/EURL-AP%20SOP%20Ruminant%20PCR%20V1.0.pdf

The primers and probes included in this kit are the same those indicated in the official method. The most important innovation included in the design of this kit is the IPC system, needed to detect false negative result.

Take into account that is necessary to increase the PCR cycles until 50 and calculate a new cut-off

10. Quality control

All products manufactured and marketed by 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
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