RapidFinder™ Quant Multi-Meat Set

SKU A24399

For testing of Food and Environmental samples only.

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1. Introduction

Identification and quantification of meat species presence in food samples is an essential step in order to improve the traceability and the control within the food supply chain, as well as a necessary quality control for handling and cleaning processes of production lines.

RapidFinder™ Quant Multi-Meat Set allows the percentage of one species (see the list of species available below) DNA in a sample to be determined with respect to total animal DNA.

The DNA quantification is performed by real time PCR using TaqMan®-MGB probes. **RapidFinder™ Quant Multi-Meat Set** contains the standard (version 2) with which the samples can be compared to determine the percentage of one species.

To determine the percentage of one species DNA versus total animal DNA present in one sample, we need to use the **RapidFinder™ Quant Multi-Meat Set** in combination with one of these kits:

RapidFinder™ Beef ID Kit	**SKU A24391**
RapidFinder™ Pork ID Kit	**SKU A24392**
RapidFinder™ Poultry ID Kit	**SKU A24397**
RapidFinder™ Chicken ID Kit	**SKU A24393**
RapidFinder™ Turkey ID Kit	**SKU A24394**
RapidFinder™ Equine ID Kit	**SKU A15570**
RapidFinder™ Sheep ID Kit	**SKU A24395**
RapidFinder™ Goat ID Kit	**IMG-175**
RapidFinder™ Fallow Deer ID Kit	**IMG-177**

2. Kit description

Sample analysis comprises two real-time PCR simultaneous processes, one of which allows the total amount of one species DNA in the sample to be quantified, and the other of which determines the amount of animal DNA present in the sample.

PCR process to determine the total amount of one species:
To perform this reaction you need to use one of the kits listed above. Each kit contains the Beef/Pork/Poultry/Chicken/Turkey/Sheep/Goat/Fallow Deer or Equine master mix that includes two primers and a TaqMan®-MGB probe labelled with FAM™ fluorophore. This reaction amplifies one specific mitochondrial DNA sequence corresponding to the species referred by the

kit. For example, in the case of TaqMan® Beef ID Kit, this reaction amplifies specifically a region of mitochondrial DNA only present in Beef.

PCR process to determine the amount of animal:
This reaction includes two primers and a TaqMan®-MGB probe labelled with the FAM™ fluorophore. The reaction specifically amplifies a highly conserved mitochondrial genomic region from animal species.

RapidFinder™ Quant Multi-Meat Set includes a plasmid DNA standard (version 2) containing a copy of each of the targets used during analysis. The standard concentration is $2x10^7$ DNA copies/µl. A comparison of the results obtained with the samples and this standard allows a relative quantification to be made and therefore the percentage of the selected species, with respect to the animal mitochondrial DNA in the sample to be calculated.

3. Limit of quantification

This kit allows relative quantifications of up to 0.05% of specific animal species to be determined with respect to total animal in a sample. Take in consideration that the relative limit of quantification varies depending on the sample analysed.

4. Kit contents and storage

The kit contents the necessary reagents to perform 48 reactions:

Reagents	Identification	Amount	Storage
Multi-Meat Master Mix	Blue pad	360 µl	-20ºC
General Master Mix	White pad	600 µl	4ºC
Multi-Meat Standard (v.2)	Blue cap	5 x 40 µl	-20ºC

5. Equipment requirements

In the following table the equipment requirements for using **RapidFinder™ Quant Multi-Meat Set** are shown:

	EQUIPMENT
1	Real-time PCR thermal cycler with detection channel for FAM™ fluorophore (520 nm)
2	Set of micropipettes (10 µl, 20 µl and 200 µl)
3	Desktop centrifuge with adaptors for 96 well PCR plates and/or 0,2 ml tubes
4	Vortex

6. Consumables required

The following table lists the consumables required when using the **RapidFinder™ Quant Multi-Meat Set**:

	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

7. Amplification reactions procedure

To quantify one animal species DNA present in a sample is necessary one of the six available animal species ID kits:

RapidFinder™ Beef ID Kit SKU A24391 Cow Master Mix General Master Mix Positive Control	**RapidFinder™ Pork ID Kit** SKU A24392 Swine Master Mix General Master Mix Positive Control	**RapidFinder™ Poultry ID Kit** SKU A24397 Poultry Master Mix General Master Mix Positive Control
RapidFinder™ Equine ID Kit Ref.: A15570 Equine Master Mix General Master Mix Positive Control	**RapidFinder™ Chicken ID Kit** SKU A24393 Chicken Master Mix General Master Mix Positive Control	**RapidFinder™ Turkey ID Kit** SKU A24394 Turkey Master Mix General Master Mix Positive Control
RapidFinder™ Sheep ID Kit SKU A24393 Sheep Master Mix General Master Mix Positive Control	**RapidFinder™ Goat ID Kit** IMG-175 Goat Master Mix General Master Mix Positive Control	**RapidFinder™ Fallow Deer ID Kit** IMG-177 Fallow Deer Master Mix General Master Mix Positive Control

With

RapidFinder™ Quant Multi-Meat Set Ref.: IMG-166 Multi-Meat Master Mix General Master Mix Multi-Meat Standard

Two absolute quantifications are performed during the course of the relative quantification of animal species, present in a sample. The first of these, determines the total amount of animal DNA present in the sample and the second determines the amount of each species DNA in the sample (Beef/Pork/Poultry/Chicken/Turkey/Sheep/Goat/Fallow Deer or Equine), depending on the kit used.

Preparation of the amplification reactions includes:

- Standard dilutions
- Negative PCR and/or extraction controls
- Sample analysis in duplicate.

The number of samples and controls to be analysed simultaneously should be taken into account when determining the amounts of reagents required. We recommend performing the calculation by assuming an extra reaction, or increasing the volume of each reagent by 10%.

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

1. Thaw a vial of Multi-Meat standard and prepare four 1:10 serial dilutions of this standard (see the figure). This process results in the quantitative standards with which the samples can be compared.

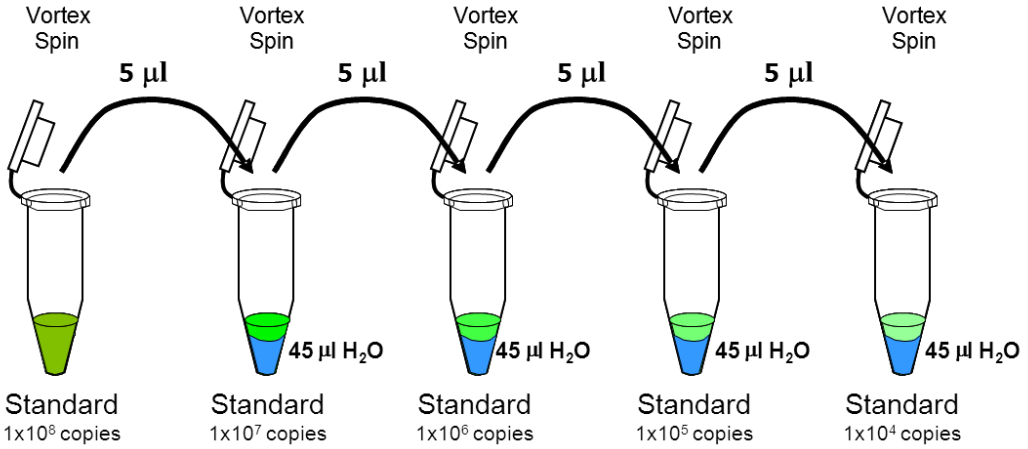

Figure 1: Five serial standard dilutions are made from Multi-Meat Standard to perform two standard curves.

2. Shake the Master Mixes included in the selected Kit (beef master mix/ pork master mix/ poultry master mix / chicken master mix / turkey master mix/ sheep master mix/ goat master mix/ fallow deer master mix or equine master mix) and **RapidFinder™ Quant Multi-Meat Set** on the vortex whilst keeping them cold.
3. For each reaction to be performed, mix 7.5 µL of Multi-Meat master mix with 12.5 µL of General master mix at one 1.5 ml tube. Shake on the vortex, then pipette 20 µL into each well or tube (see the example for three samples in figure 2).
4. For each reaction to be performed mix 7.5 µL of beef master mix / pork master mix / poultry master mix / chicken master mix/ turkey master / sheep master mix / goat master mix/ fallow deer master mix or equine master mix with 12.5 µL of General master mix at one 1.5 ml tube. Shake on the vortex, then pipette 20 µL into each well or tube.
5. Add 5 µl of each sample DNA (10 ng/µL) to the corresponding wells:

a. Beef/pork/poultry/chicken/turkey/sheep/goat/fallow deer or equine amplification reactions and,
b. Animal amplification reactions

6. Add 5 µl of each standard dilution to the corresponding wells:
   a. Beef/pork/poultry/chicken/turkey/sheep/goat/ fallow deer or equine amplification reactions and,
   b. Animal amplification reactions

7. Add 5 µl of each control (negative control and DNA extraction control) to the corresponding wells:
   a. Beef/pork/poultry/chicken/turkey/sheep/goat/fallow deer or equine amplification reactions and,
   b. Animal amplification reactions

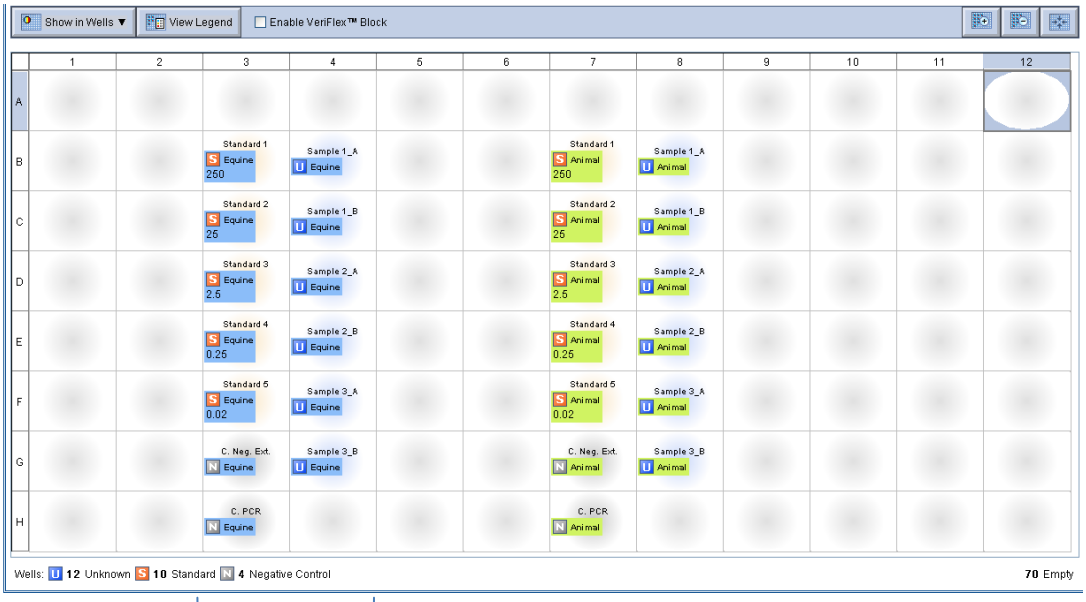

Figure 2: Proposed design analysis for 3 samples obtained from the same DNA extraction round. In this case we use the RapidFinder™ Equine ID Kit in combination with ***RapidFinder™ Quant Multi-Meat Set****.*

8. Seal the plate with optical film and spin.
9. Load the plate into a thermal cycler and then perform a run using the conditions showed in the next section.

Note: We **strongly recommend** making each sample analysis in duplicate. We also 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 recommend using a **PCR negative control** for each PCR run (this tube contains all PCR reagents and water instead of DNA sample).

8. PCR Amplification program

We recommend 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.

9. Analysis of results

Before analysing the sample results, it should be confirmed that the results obtained with the different controls are as expected:

- **Negative controls:** Amplification must be only detected in the VIC® channel for animal species reaction of amplification. No amplification should be detected in either the reaction corresponding to Animal. Amplification in a negative control would indicate the presence of contamination and therefore that the assay should be repeated.

- **Multi-Meat Standard:** Amplification should be detected for the five points corresponding to the Multi-Meat standard and the five points corresponding to the beef/ pork / poultry/ turkey/ chicken/ sheep/ goat/ fallow deer or equine standard. Furthermore, the curves obtained using the standard points should meet the following requirements:

- The efficiency of the curve should be between 86% and 110%.
- The slope of the curve should be between -3.1 and -3.7.
- The correlation coefficient (R^2) should be greater than 0.98.

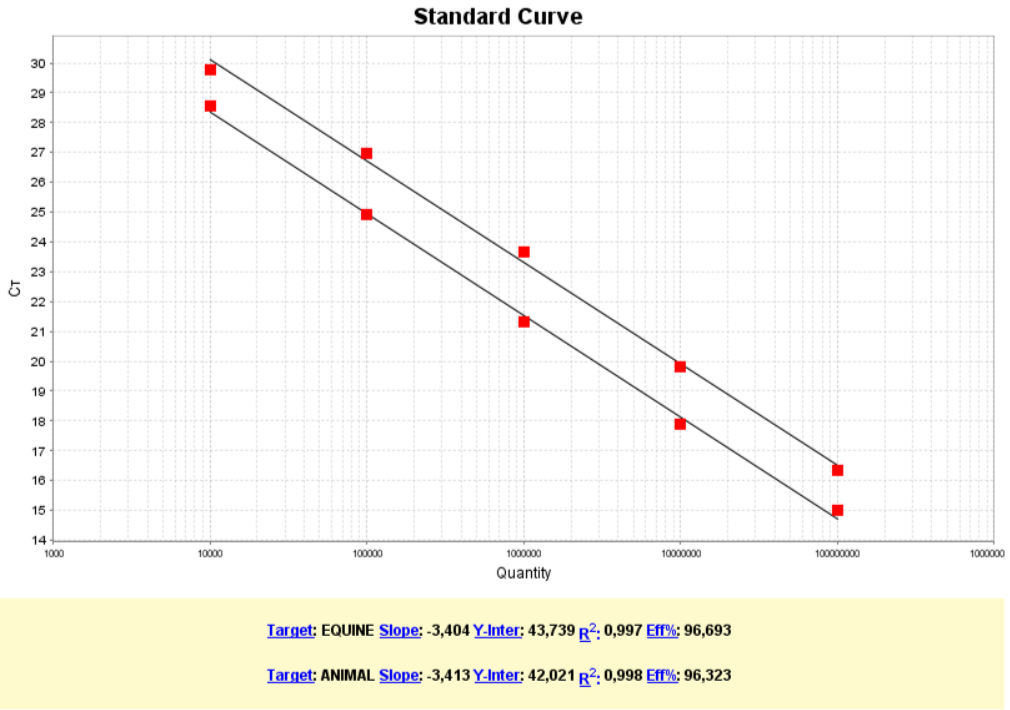

Figure 3: Standard curves for Multi-Meat and, in this case, equine targets. Red dots represent the dilutions of the standard.

Once the controls have been verified, the results obtained with the samples can be analysed. If duplicated have been performed, the results for both replicates should be similar.

Two results are possible for each amplification reaction of both beef/pork/poultry/turkey/chicken/sheep/goat/ fallow deer or equine DNA and Multi-Meat DNA:

- **Quantifiable:** Amplification is detected in the sample to an extent greater than the last point on the curve. When the amplification Ct for the sample is interpolated between the values for the standard points, the quantitative result can be considered to be reliable and can be used to calculate the percentage of beef/ pork/ turkey/ chicken/ sheep/ goat/ fallow deer or equine DNA.

- **Not Quantificable:** No amplification is detected in the sample or the amplification detected is lower than the last point on the curve.

The following formula should be used to calculate the percentage of beef/pork/poultry/turkey/chicken/sheep/goat or equine (species) DNA with:

$$\% \text{ Species DNA} = \frac{\text{Species DNA x 100}}{\text{Animal DNA}}$$

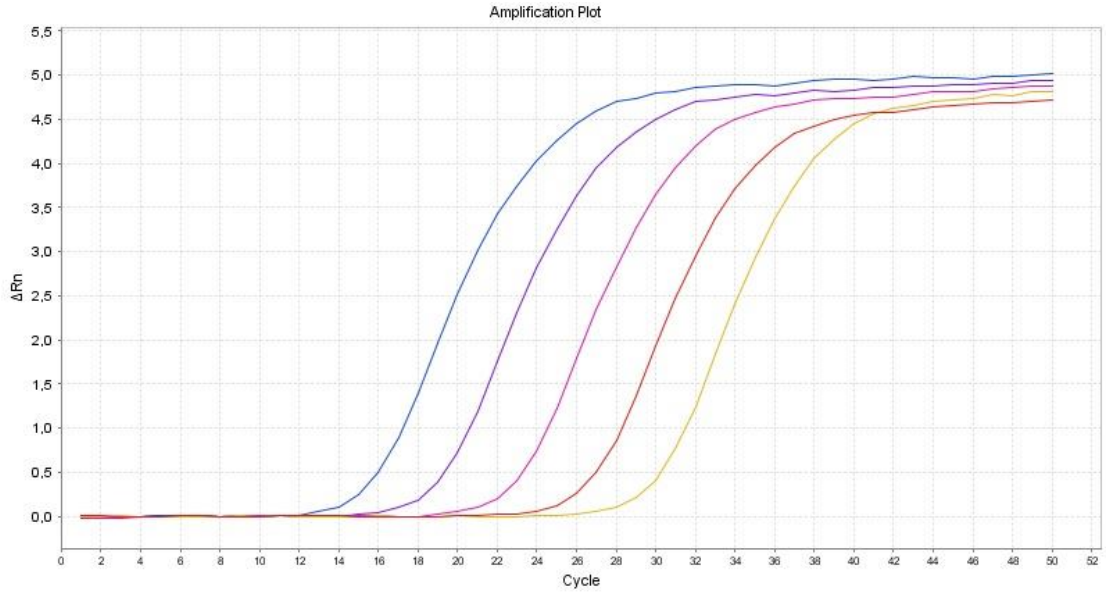

Figure 4: Amplification curves for each of the dilutions of the Multi-Meat standard (version 2) using Multi-Meat master mix

The following table lists the results that can be obtained upon analysis of a sample and a recommendation about how they should be interpreted:

Animal	Animal Species*	Interpretation
Quantifiable	Not quantifiable	No specific animal species detected in the sample or the amount of specific animal species detected in the sample is lower than the limit of quantification
Quantifiable	Quantifiable	The amount of specific animal species DNA with respect to total animal DNA in the sample is X%
Not quantifiable	Not quantifiable	The amounts of specific animal species and animal DNA detected in the sample are lower than the limit of quantification

*One of the follow animal species: beef, pork, poultry, turkey, chicken, sheep, goat or equine.

10. Quality Control

All products manufactured and marketed by the Institute of Medical Genomics are submitted to a rigorous quality control process. The **RapidFinder™ Quant Multi-Meat Set** has passed all 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.

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