RapidFinder™ Quant Equine Set

Part No: A15579

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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 Equine Set allows the percentage of horse DNA in a sample to be determined with respect to total animal DNA.

Equine DNA quantification is performed by real time PCR using TaqMan®-MGB probes and contains the standard with which the samples can be compared to determine the percentage of horse.

To determine the percentage of equine DNA versus total animal DNA present in one sample, we need to use this kit in combination with **RapidFinder™ Equine ID Kit (Ref. A15570)**

2. Kit description

Sample analysis comprises two real-time PCR simultaneous processes, one of which allows the total amount of horse 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 horse:
To perform this reaction you need to use **RapidFinder™ Equine ID Kit** (Ref. A15570). This kit contains the equine master mix that includes two primers and a TaqMan®-MGB probe labelled with FAM fluorophore. This reaction specifically amplifies one mitochondrial DNA sequence of *Equus caballus*.

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 Equine Set includes a standard consisting of a purified horse DNA at 50 ng/ul. A comparison of the results obtained with the samples and this standard allows a relative quantification to be made and therefore the percentage of horse mitochondrial DNA 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 horse 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
Animal Master Mix	Blue pad	378 µl	-20ºC
General Master Mix	White pad	630 µl	4ºC
Equine Standard	Red cap	5 x 40 µl	-20ºC

5. Equipment requirements

In the following table the equipment requirements for using **RapidFinder™ Quant Equine 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 Equine 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 the horse DNA present in a sample, is necessary to use the next two kit references:

- **RapidFinder™ Equine ID Kit** (48 rxn), *Ref.: A15570*
  - Equine Master Mix
  - General Master Mix
  - Positive Control

- **RapidFinder™ Quant Equine Set** (48 rxn), *Ref.: A15579*
  - Animal Master Mix
  - General Master Mix
  - Equine Standard

Two absolute quantifications are performed during the course of the relative quantification of horse 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 horse DNA in the sample.

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 Equine 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.

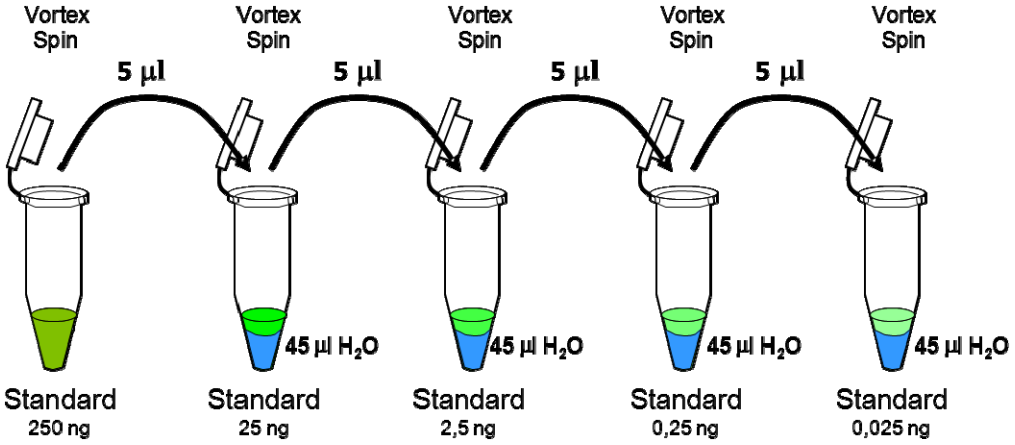

Figure 1: Five serial standard dilutions are made from equine master mix to perform two standard curves.

2. Shake the Master Mixes included in the **RapidFinder™ Equine ID Kit** and **RapidFinder™ Quant Equine Set** on the vortex whilst keeping them cold.
3. For each reaction to be performed, mix 7,5 µL of Animal 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 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 (equine amplification reactions and animal amplification reactions)
6. Add 5 µl of each standard dilution to the corresponding wells (equine amplification reactions and animal amplification reactions)
7. Add 5 µl of each control (negative control and DNA extraction control) to the corresponding wells (equine amplification reactions and animal amplification reactions)

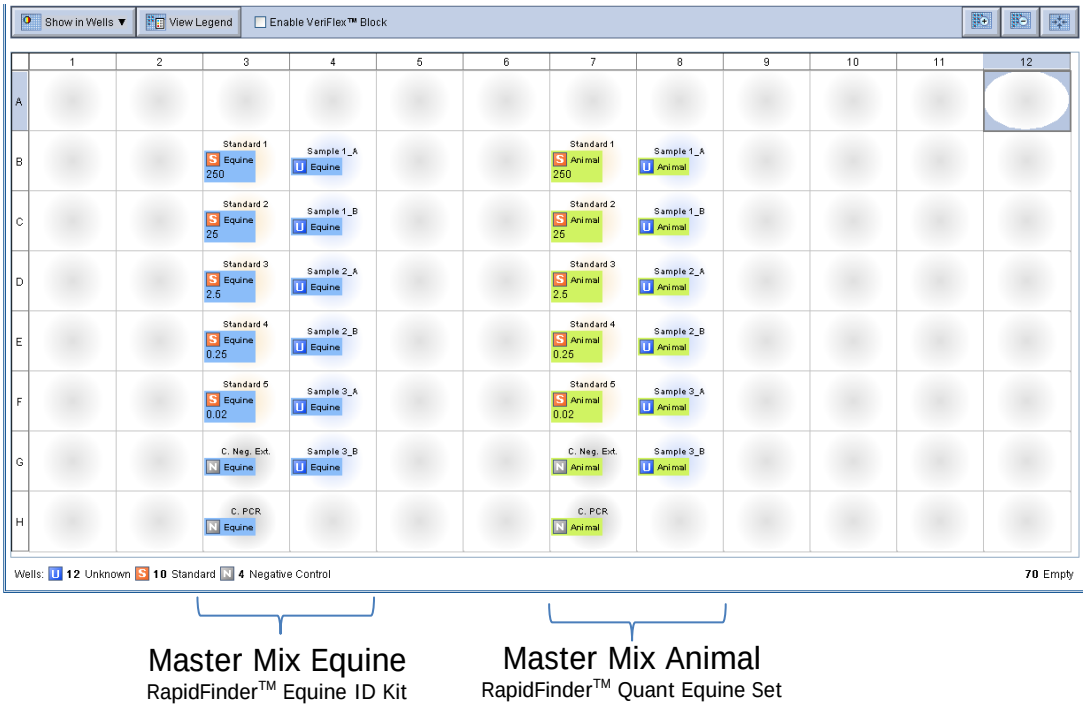

Figure 2: Proposed design analysis for 3 samples obtained from the same DNA extraction round.

8. Seal the plate with optical film and spin
9. Load the plate into a thermal cycler, 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 equine 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.

- **Equine Standard:** Amplification should be detected for the five points corresponding to the animal standard and the five points corresponding to the equine standard. Furthermore, the curves obtained using the standard points should meet the following requirements:

  - The efficiency of the curve should be between 90% and 105%.
  - The slope of the curve should be between -3.1 and -3.9.
  - The correlation coefficient (R^2) should be greater than 0.98

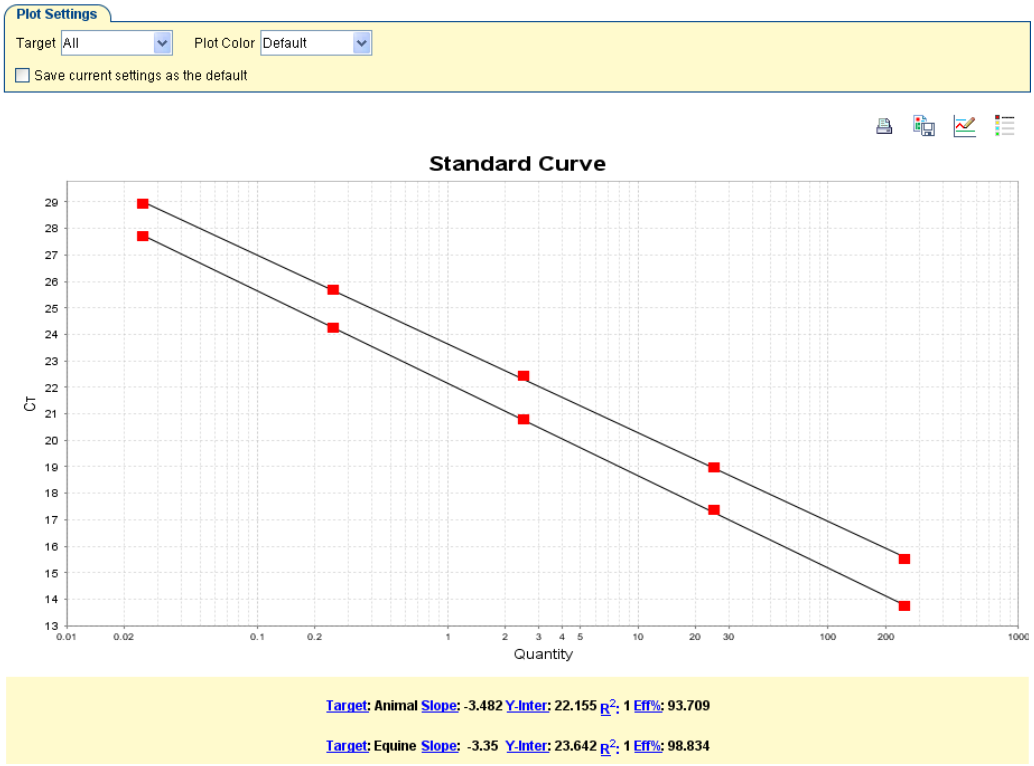

Figure 3: Standard curves for animal and equine targets. Red dots represent the dilutions of the standard.

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 equine DNA and animal 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 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 Equine DNA with:

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

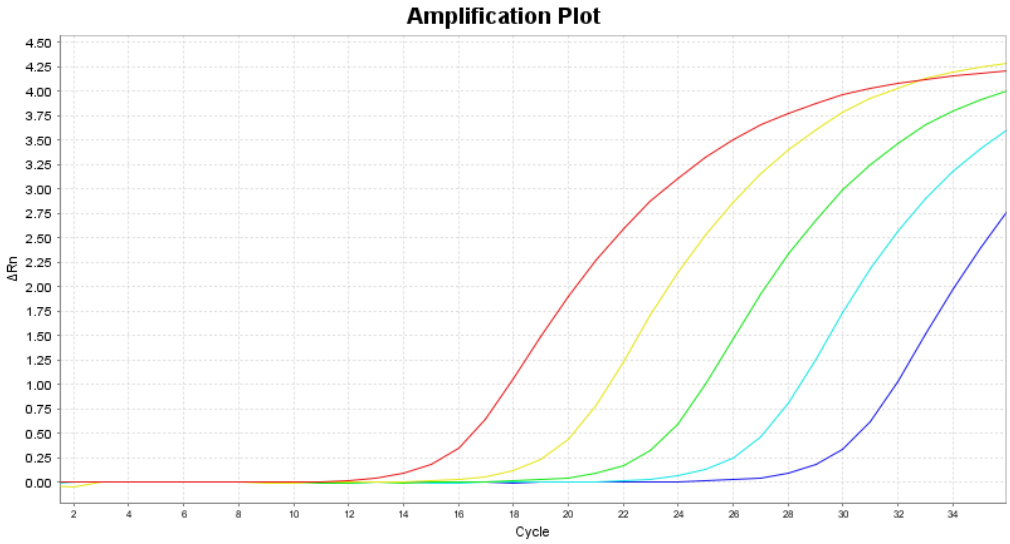

Figure 4: Amplification curves for each of the dilutions of the standard Equine using Animal master mix

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

10. Quality Control

All products marketed by the Institute of Medical Genomics are submitted to a rigorous quality control process. The **RapidFinder™ Quant Equine 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.

11. Warranties and liabilities

Instituto de Medicina Genómica guarantees that all its products are free from defects, both in the used materials as in its manufacturing process. This warranty is extended to a period of one year from the date of shipment of the product, provided that storage conditions specified in this Manual have been observed. **Our products are designed for its use in testing of food and environmental samples.** The user of the product is responsible for validating the usefulness of the protocol proposed by Instituto de Medicina Genómica. These protocols are considered only guidelines. Instituto de Medicina Genómica does not offer any other warranty, either expressed or implied, which extend beyond the proper functioning of the components of this set. The only obligation of Instituto de Medicina Genómica, for the foregoing warranties, will be the replacement of the product or return the purchase price thereof, as desired by the customer, provided that proves the existence of a defect in materials, or in the development of the products. The Instituto de Medicina Genómica will not be responsible for any damages, direct or indirect, resulting in economic losses or damages resulting from the use of this product by the buyer or user.

12. Customer Support

For any inquiries over applications of this product or its protocols, please contact our Technical Department:

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