imegenagro
Food Safety

User Guide

imegen™ Quant Species Kit

DNA quantitation of an animal species against total DNA animal by real-time PCR

REF : IMG-272

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All products sold by the imegen are subjected to rigorous quality control. The ***imegen™ Quant Species kit*** *has passed all internal validation tests, ensuring the reliability and reproducibility of each assay.*

For any questions about the applications of this product or its protocols, please contact our Technical Department:

Phone number: *963 212 340*
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1. Product information

Kit description

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.

***imegen-Quant Species Kit** 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. **imegen-Quant Species Kit** contains the standard 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 **imegen-Quant Species Kit** in combination with one of these kits:*

imegen-Beef ID Kit	*IMG-264*
imegen-Pork ID Kit	*IMG-265*
imegen-Poultry ID Kit	*IMG-270*
imegen-Chicken ID Kit	*IMG-266*
imegen-Turkey ID Kit	*IMG-267*
imegen-Equine ID Kit	*IMG-263*
imegen-Sheep ID Kit	*IMG-268*
imegen-Goat ID Kit	*IMG-175*
imegen-Fallow Deer ID Kit	*IMG-177*

Content and storage of the kit

Sample analysis comprises two real-time PCR simultaneous processes:

- *One of them allows the total amount of one species DNA in the sample*
- *The other, allows the total amount of animal DNA present in the sample*

To perform the reaction to determine the total DNA amount of one species 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.

To perform the reaction to determine the DNA amount of animal in the sample you need to use this kit which 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.

***imegen-Quant Species Kit** includes a plasmid DNA standard 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.*

The kit contents the necessary reagents to perform 48 reactions:

Reagents	Color	Amount	Storage
Species Master Mix	Blue pad	360 µL	-20ºC
General Master Mix	White pad	600 µL	4ºC
Species Standard	Blue cap	5 x 40 µL	-20ºC

Table 1.** Kit components and storage temperature of **imegen-Quant Species Kit

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Equipment and material required but not supplied

In the following table the equipment requirements for using ***imegen-Quant Species 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

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

Quantification limit

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.

2. Real time PCR

PCR reactions preparation

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

imegen-Beef ID Kit *IMG-264* *Cow Master Mix* *General Master Mix* *Positive Control*	***imegen-Pork ID Kit*** *IMG-265* *Swine Master Mix* *General Master Mix* *Positive Control*	***imegen-Poultry ID Kit*** *IMG-270* *Poultry Master Mix* *General Master Mix* *Positive Control*
imegen-Equine ID Kit *IMG-263* *Equine Master Mix* *General Master Mix* *Positive Control*	***imegen-Chicken ID Kit*** *IMG-266* *Chicken Master Mix* *General Master Mix* *Positive Control*	***imegen-Turkey ID Kit*** *IMG-267* *Turkey Master Mix* *General Master Mix* *Positive Control*
imegen-Sheep ID Kit *IMG-268* *Sheep Master Mix* *General Master Mix* *Positive Control*	***imegen-Goat ID Kit*** *IMG-175* *Goat Master Mix* *General Master Mix* *Positive Control*	***imegen-Fallow Deer ID Kit*** *IMG-177* *Fallow Deer Master Mix* *General Master Mix* *Positive Control*

With:

imegen-Quant Species Kit *IMG-272* *Species Master Mix* *General Master Mix* *Species 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*

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 a vial of Species 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.*

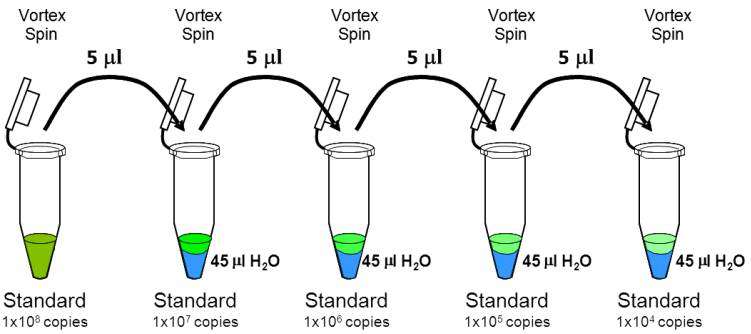

Figure 1: Four serial standard dilutions are made from Species Standard to perform two standard curves

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2. *Vortex each reagent and keep cold.*
3. *Add into a 1.5 mL tube, the following reagents:*

Reagents	Amount per reaction
Species Master Mix	7.5 µL
General Master Mix	12.5 µL

***Table 3**. Reagents amount per reaction*

Add into another 1.5 mL tube, the following reagents:

Reagents	Amount per reaction
Specific species Master Mix	7.5 µL
General Master Mix	12.5 µL

***Table 4**. Reagents amount per reaction*

4. *Vortex and spin the 1.5 mL tubes and dispense 20 µl per well or tube of 0.2 ml (see the example for three samples in figure 2).*
5. *Add 5 µl of each sample DNA (10 ng/µL) to the corresponding wells:*
    a. *Specific species reactions and,*
    b. *Animal amplification reactions*
6. *Add 5 µl of each standard dilution to the corresponding wells:*
    a. *Specific species reactions and,*
    b. *Animal amplification reactions*
7. *Add 5 µl of each control (negative control and DNA extraction control) to the corresponding wells:*
    a. *Specific species reactions and,*
    b. *Animal amplification reactions*
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.*

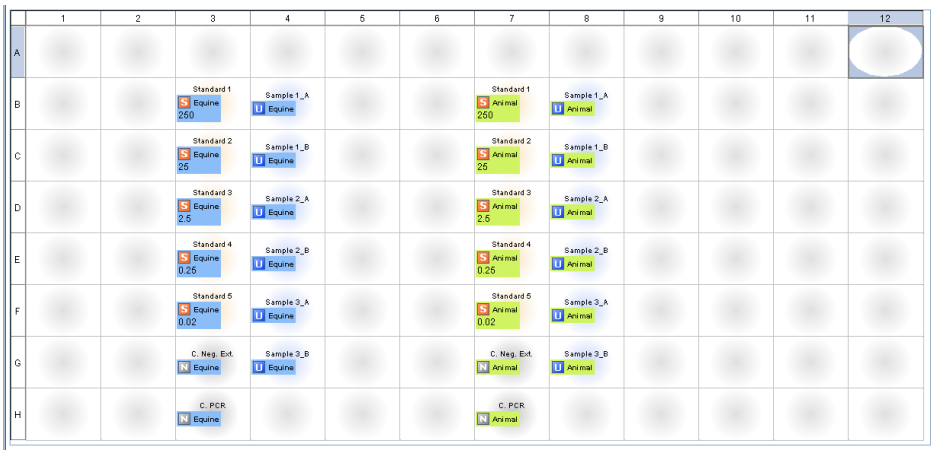

*Figure 2: Proposed design analysis for 3 samples obtained from the same DNA extraction round. In this case we use the imegen-Equine ID Kit in combination with **imegen-Quant Species kit**.*

** 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.*

PCR amplification program

This kit is compatible with the Real-time PCR platforms 7500 FAST, StepOne Real-Time PCR System (Thermo Scientific), and any other Real-time PCR platforms equipped with FAM and VIC channels.

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Probes	Receptor	Quencher
Animal-P	FAM™	MGB
Specific Species-P	FAM™	MGB
IPC-P	VIC®	MGB

Table 5. *Probes information*

The following instructions should be taken into account in order to setup the amplification program:

- *Reaction volume: 25 µL*
- *Targets: FAM and VIC*
- *In case the quencher has to be defined, select MGB for all probes. If the real time PCR system does not take into account the quenchers, select only the receptors (FAM and VIC).*
- *If the real time PCR system is a 7500 Fast or a StepOne Real-Time PCR system (Thermo Scientific) select Quantitation- Standard curve as a type of experiment and include ROX™ as a reference.*
- *Ramp rate: standard*

- *Optimal program:*

Fields	Step 1 Enzyme activation	Step 2 PCR	
Cycle Number	1 initial cycle	36 cycles	
		Denaturation	Primers binding/Extention
Temperature	95°C	95°C	60°C
Time	10 minutes	15 seconds	1 minute*

***Table 5.** Optimal PCR program*

**Fluorescence detection*

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.

3. Results analysis

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.*

- ***Species Standard:*** *Amplification should be detected for the five points corresponding to the Species standard and the five points corresponding to the beef/ pork / poultry/ turkey/ chicken/ sheep/ goat/ fallow deer or equine standard.*

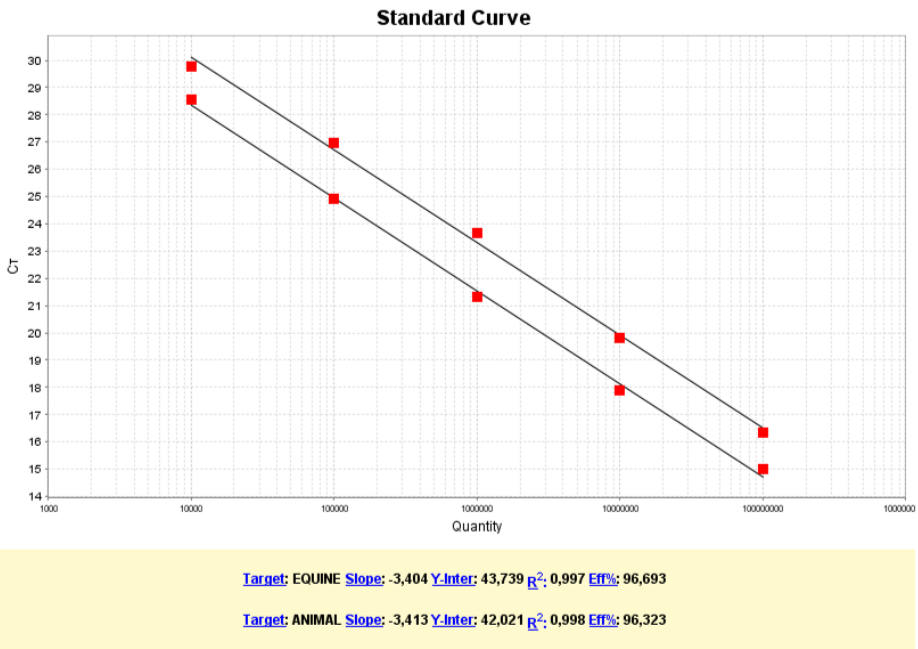

Figure 3: Standard curves for animal and, in this case, equine targets. Red dots represent the dilutions of the standard.

Furthermore, the curves obtained using the standard points should meet the following requirements:

- *The efficiency of the curve should be between 80% and 110%.*
- *The slope of the curve should be between -3.1 and -3.9.*
- *The correlation coefficient (R^2) should be greater than 0.98.*

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 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 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:

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

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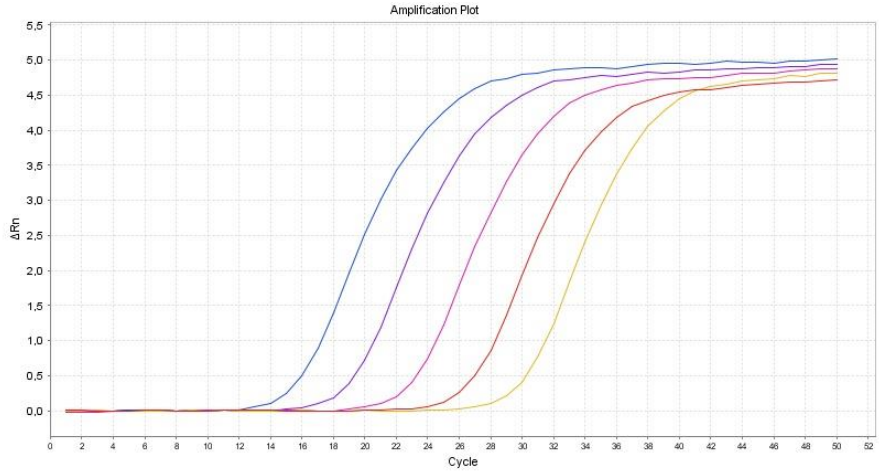

Figure 4: Amplification curves for each of the dilutions of the Species Standard using Species master mix

The following table shows the interpretation that should be done from the obtained result:

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

***Table 8.** Possible results and their interpretation*

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

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