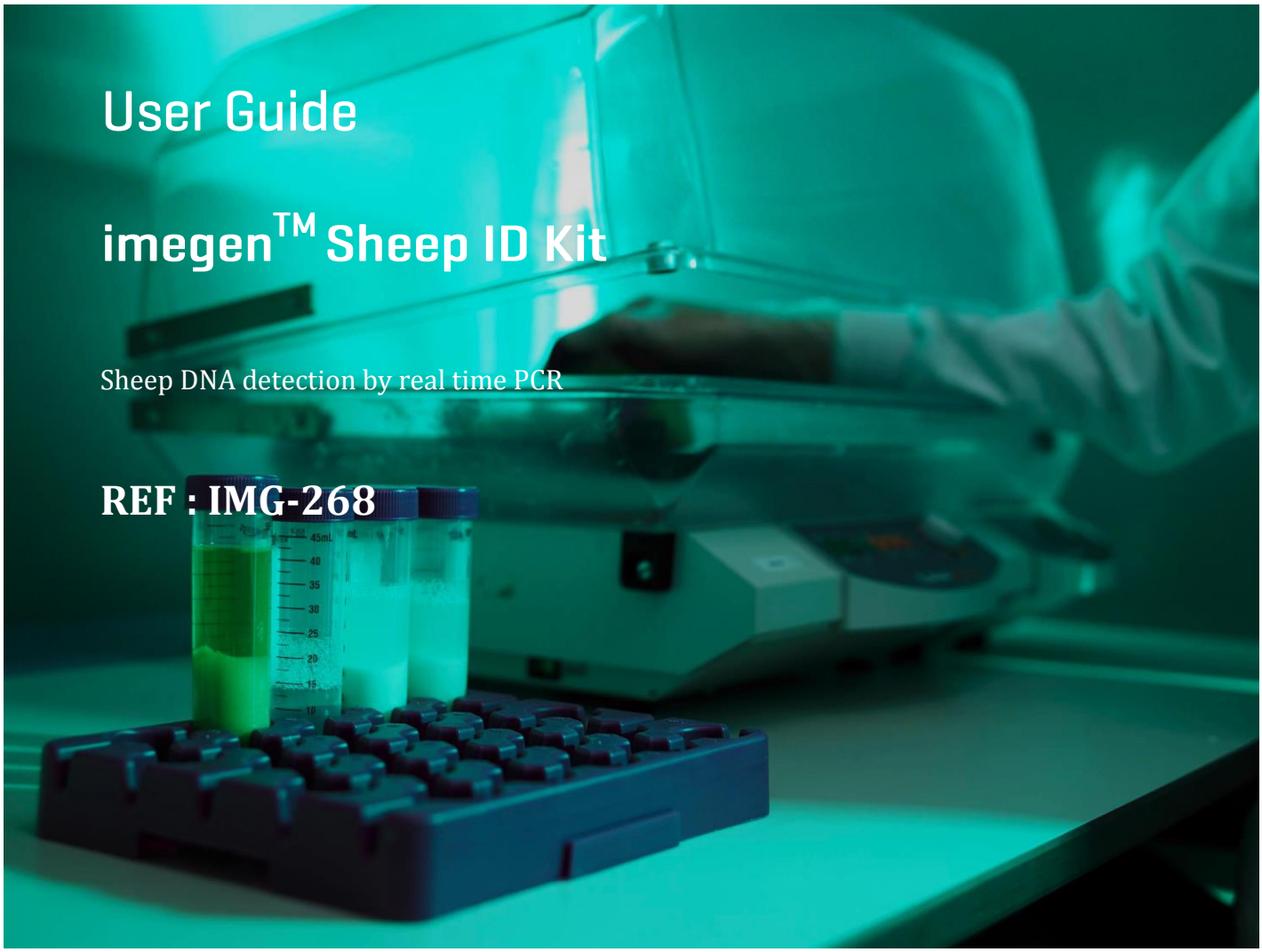
imegenagro
Food Safety



User Guide

imegen™ Sheep ID Kit

Sheep DNA detection by real time PCR

REF : IMG-268

Instituto de Medicina Genómica SL
Agustín Escardino 9,
Parc Científic de la Universitat de València
46980 Paterna (Valencia, España)
+34 963 212 340 - info@imegen.es

imegen.es

Rev. 3

The information in this guide is subject to change without notice.

Imegen guarantees that its products are free from defects, both in used materials as in its manufacturing process. This warranty is extended to the expiration date, as long as the storage conditions specified in this manual are met. ***Our products are designed for use in research.*** *The user of the product is responsible for validating the usefulness of the protocol proposed by imegen. These protocols are considered a guide only. Imegen does not offer any other warranty, express or implied, which extend beyond the proper functioning of the components of this set. Imegen sole obligation in respect of the preceding guarantees, will be to replace the product or return the purchase price thereof, as desired by the customer, as long as the existence of a defect in the materials test, or in the manufacture of its products. Imegen will not be responsible for any damage, direct or indirect, resulting in economic losses or damages resulting from the use of this product by the purchaser or user.*

All products sold by the imegen are subjected to rigorous quality control. The ***imegen™ Sheep ID 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*
e-Mail: *info@imegen.es*

*****imegen™*** *is a trademark of Instituto de Medicina Genómica, registered as imegen® in Spain*

Instituto de Medicina Genómica SL
Agustín Escardino 9,
Parc Científic de la Universitat de València
46980 Paterna (Valencia, España)
+34 963 212 340 - info@imegen.es

imegen.es

imegenagro

Index

Instituto de Medicina Genómica SL
Agustín Escardino 9,
Parc Científic de la Universitat de València
46980 Paterna (Valencia, España)
+34 963 212 340 - info@imegen.es

imegen.es

1. Product information

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.

***imegen-Sheep ID Kit** allows determining the presence of DNA of Sheep (Ovis aries) in any food.*

Sheep 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 Sheep. 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 Sheep meat and other species. **imegen-Sheep ID Kit** can detect blends containing a percentage below 0.1% (w/w) of Sheep 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 **Food Extraction Kit** (Part No: IMG-262).*

Instituto de Medicina Genómica SL
Agustín Escardino 9,
Parc Científic de la Universitat de València
46980 Paterna (Valencia, España)
+34 963 212 340 - info@imegen.es

imegen.es

Content and storage of the kit

The kit contains needed reagents to perform the real time PCR reactions:

- *Amplification primers for the PCR systems.*
- *The system contains two probes. One of them labelled with FAM™ for the specific Sheep DNA detection and other labelled with VIC® for the IPC detection.*
- *Sheep Positive control (0.1% of Ovis aries).*

The kit contents the necessary reagents to perform 48 reactions:

Reagents	Color	Amount	Storage
Sheep Master Mix	Purple pad	360 µL	-20ºC
General Master Mix	White pad	600 µL	4ºC
Positive Control	Purple cap	60 µL	-20ºC

Table 1.** Kit components and storage temperature of **imegen Sheep ID Kit

Equipment and material required but not supplied

*In the following table the equipment requirements for using **imegen-Sheep 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

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

Detection and quantification limits

The detection limit is the minimal amount of DNA that can be detected. This limit has been estimated using standard manufactured in our laboratory.

***imegen-Sheep ID Kit** can detect blends containing a percentage above 0.01% of Sheep DNA. The limit of detection in processed samples varies depending on the composition and food processing.*

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	D
Goat	ND
Horse	ND
Buffalo	ND
Chicken	ND
Deer	ND
Beef	ND
Pork	ND
Turkey	ND
Fish	ND
Duck	ND
Ostrich	ND
Goose	ND
Human	ND

Table 2**. Specificity of **imegen Sheep ID Kit

D: Detected

ND: Not Detected

Instituto de Medicina Genómica SL
Agustín Escardino 9,
Parc Científic de la Universitat de València
46980 Paterna (Valencia, España)
+34 963 212 340 - info@imegen.es

imegen.es

2. Real time PCR

PCR reactions preparation

***imegen-Sheep ID Kit** is designed to determine, in a single PCR reaction, the presence or absence of Sheep DNA and the internal positive control.*

We recommend using, the positive control included in this kit for each run.

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 Sheep Master Mix and the Positive Control vial.*
2. *Vortex each reagent and keep cold.*
3. *Add into a 1.5 mL tube, the following reagents:*

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

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

4. *Vortex and spin the 1.5 mL tube and dispense 20 µl per well or tube of 0.2 ml.*
5. *Add 5 µl of each DNA sample at 10 ng/µl, into the appropriate wells. We recommend making each sample analysis in duplicate.*
6. *Add 5 µl of Positive Control and Negative Controls* into the appropriate wells.*

7. *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.*

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.

Probes	Receptor	Quencher
Sheep-P	FAM™	MGB
IPC-P	VIC®	MGB

***Table 4.** 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 samples results, it should be checked if obtained results in controls are 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 Sheep DNA (FAM™) is detected, because the PCR reagents are exhausted before amplification of the IPC begins.

Sheep

Amplification in the FAM™ channel indicates presence of Sheep 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 Ovis aries DNA.

In samples where no amplification in the FAM™ channel is seen, we can conclude that no Sheep 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:

Sheep Master Mix		Interpretation
Sheep	IPC	
-	+	No Sheep DNA is detected
+	+	Sheep DNA is detected
-	-	PCR inhibitors presence in the sample*
+	-	Sample with big amount of Sheep DNA

***Table 6.** Results interpretation*

* *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	Sheep Master Mix		Interpretation
	Sheep	IPC	
Positive control	+	+	Expected result
	-	-	PCR Amplification Failure[1]
Extraction Negative Control	-	+	Expected result
	+	+	Contamination in the Sheep DNA extraction procedure[2]
PCR Negative Control	-	+	Expected result
	+	+	PCR contamination with Sheep DNA[3]

Table 7. *Possible results and their interpretation*

Recommendations:

[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 Sheep 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 Sheep 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.*