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Food Safety

User Guide

imegen™ Beef Sexing ID Kit

Beef sexing by real time PCR

REF : IMG-183

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Rev. 2

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All products sold by the Imegen are subjected to rigorous quality control. The ***imegen™-Beef Sexing 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:

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1. Product information

Kit description

In order to ensure the possibility of compliance of the Commission Regulation (EC) Nº 765/2002 of 3 May 2002, relative to the collection of samples and the adoption of certain detailed rules in connection with physical checks on boneless beef cuts qualifying for export refunds, it has been developed a beef sexing kit.

imegen-Beef Sexing ID Kit *allows the beef sexing through DNA analyses extracted from bovine meat.*

Beef sexing is done by real time PCR using two TaqMan®-MGB probes. One of them, labelled with FAM™ dye, specifically detects Y chromosome DNA sequence of males. The second probe is labelled with VIC® and detects X chromosome DNA sequence, which also allows ruling out inhibitors in the sample and check the correct functioning of the assay due to as males as females contain X chromosome. Thus, it has to have always DNA X-chromosome amplification.

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 labelled with FAM™ and VIC® fluorophores which allow the differentiation between beef male and female.*
- *Positive control for the X-allele and Y-allele analysed in this kit.*

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The kit contents the necessary reagents to perform 48 reactions:

Reagents	Color	Amount	Storage
Beef Sexing Master Mix	Red pad	360 µl	-20ºC
General Master Mix	White pad	600 µl	4ºC
Positive Control	Red cap	60 µl	-20ºC

Table 1. *Kit components and storage temperature of* ***imegen-Beef Sexing ID Kit***

Equipment and material required but not supplied

In the following table the equipment requirements for using ***imegen-Beef Sexing 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

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2. Real time PCR

PCR reactions preparation

***Imegen-Beef Sexing ID Kit** is designed to identify, in a single PCR reaction, the sex of the bovine from which proceeds the meat.*

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 Beef Sexing 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
Beef Sexing Master Mix	7.5 µL
General Master Mix	12.5 µL

***Table 2.** 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
X-probe	VIC®	MGB
Y-probe	FAM™	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	40 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, 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® and both Cts have to be similar.*
- ***Negative controls**: The result must always be negative in all amplification reactions, both in the FAM™ channel as VIC®.*

X Chromosome amplification

It must be checked that the X chromosome DNA [VIC®] is positive in all samples. A negative result in the amplification of the X chromosome indicates the presence of inhibitors in the sample or the PCR failure.

Y Chromosome amplification

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

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:

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Controls	Beef Sexing Master Mix		INTERPRETATION
	Y Chromosome	X Chromosome	
Positive Control	+	+	Expected result
	-	-	PCR Amplification Failure[1]
Extraction Negative Control	-	-	Expected result
	-	+	Female DNA contamination in the meat DNA extraction procedure[2]
	+	+	Male DNA contamination in the meat DNA extraction procedure[2]
PCR Negative Control	-	-	Expected result
	-	+	PCR contamination with female bovine DNA[3]
	+	+	PCR contamination with male bovine DNA[3]

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 meat 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 bovine 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.*

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:

Beef Sexing Master Mix		INTERPRETATION
Y chromosome	X chromosome	
-	+	Female DNA is detected
+	+	Male DNA is detected
-	-	PCR inhibitors presence in the sample*

[*] *If the control reactions are correct and in the samples are observed these results, presence of inhibitors in the sample is detected. We recommend repeating DNA extraction. If the problem persists, please contact our technical department.*