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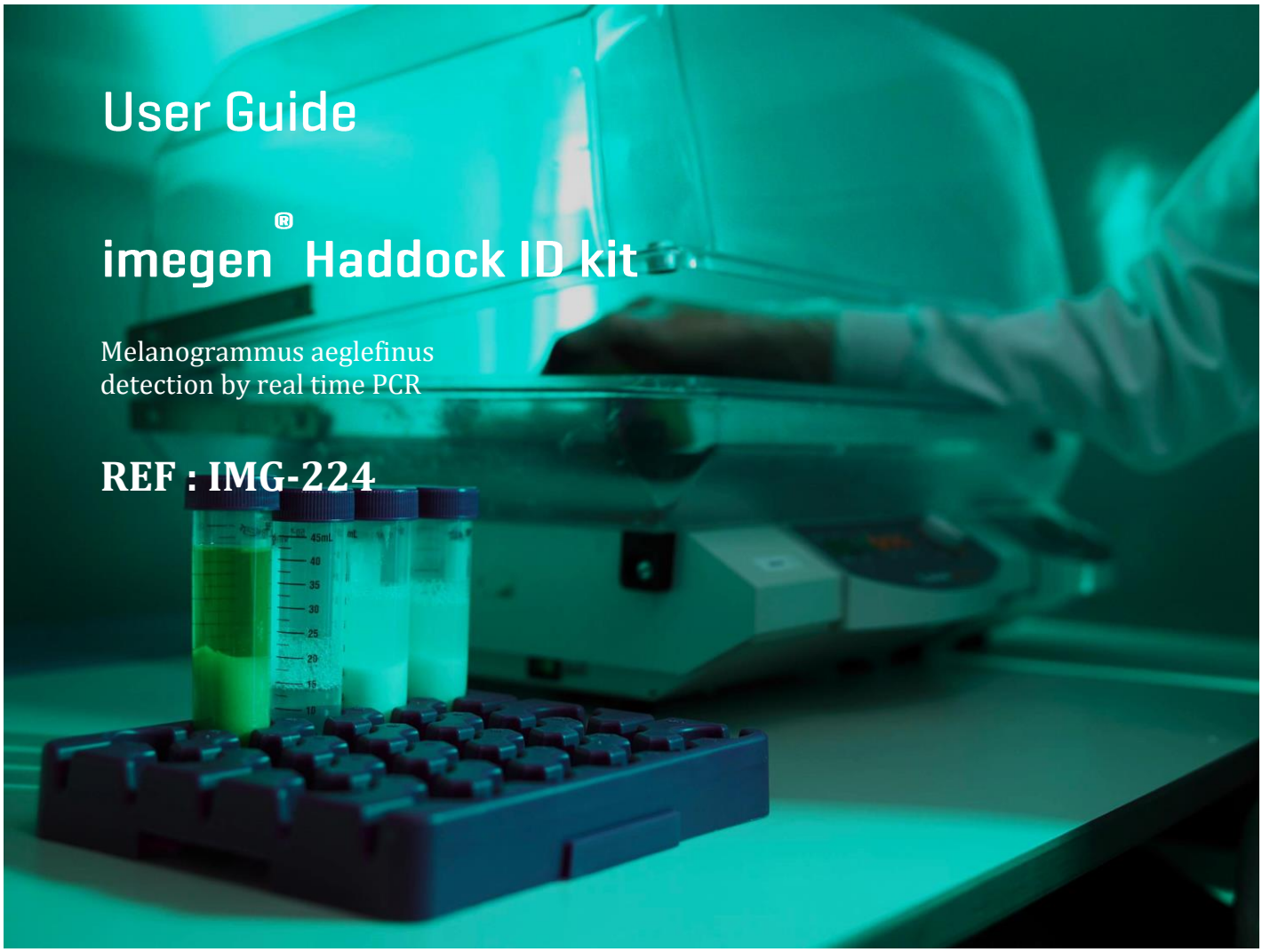

User Guide

imegen® Haddock ID kit

Melanogrammus aeglefinus
detection by real time PCR

REF : IMG-224

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All products sold by the Imegen are subjected to rigorous quality control. The ***imegen® Haddock 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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Index

1. Product information 4
   - Kit description 4
   - Content and storage of the kit 4
   - Equipment and material required 5

2. Real time PCR 7
   - PCR reactions preparation 7
   - PCR amplification program 8

3. Analysis of results 10

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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® Haddock ID Kit** allows determining the presence of DNA of Melanogrammus aeglefinus in any food.*

Melanogrammus aeglefinus DNA detection is done by real time PCR using TaqMan®-MGB probes. One of them, labelled with FAM™ dye, specifically detects one mitochondrial DNA sequence of Melanogrammus aeglefinus. 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 Melanogrammus aeglefinus meat and other species. **Imegen® Haddock ID Kit** can detect blends containing a percentage over 0.01% of Melanogrammus aeglefinus 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 **GMO Extraction Kit** (Part No: 4466336).*

Content and storage of the kit

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

- *Amplification primers for the PCR system.*

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- *Two probes labelled with FAM™ and VIC® fluorophores, to detect the specific species and the Internal Positive Control (IPC).*
- *Haddock Positive Control (0,1% of Melanogrammus aeglefinus).*
- *2X General Master Mix.*

The kit contents the necessary reagents to perform 48 reactions with each PCR system:

Reagents	Color	Amount	Storage
Haddock Master-Mix	Purple pad	360 µl	-20ºC
Haddock positive control	Purple cap	60 µl	-20ºC
General Master-Mix	White pad	600 µl	4ºC

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

This kit has been proved and is compatible with the following real time PCR platforms: 7500 FAST and StepOne Real-Time PCR System (Thermo Scientific).

Equipment and material required

*In the following table the equipment requirements for using **Imegen® Haddock ID Kit** are shown:*

Equipments	
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 caps for 0.2 ml tubes
3	Disposable micropipette filter tips (10 µL, 20 µL and 200 µL)
4	1.5 mL sterile tubes
5	Powder-free latex gloves

2. Real Time PCR

PCR reactions preparation

A PCR master mix should be prepared in order to perform the analysis with ***imegen® Haddock ID kit****:*

The PCR Master Mix contains:

- *Haddock Master-Mix*
- *General Master-Mix (2X)*

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

1. *Thaw the Haddock Master-Mix, Haddock Positive Control and samples DNA.*
2. *Vortex each reagent and keep cold.*
3. *Add into a 1.5 mL tube, one for each PCR master mix preparation, the following reagents (table 2). To estimate the amount of necessary reagents, we recommend make calculations taking into account the number of samples to be simultaneously analysed, and then considering one more reaction, or increase a 10% the volume of each reagent.*

Reagents	Amount per reaction
Haddock Master-Mix	**7.5 µL**
General Master Mix	**12.5 µL**

Table 2*. Reagents amount per reaction in 7500 FAST or StepOne*

4. *Vortex the PCR Master Mixes tubes and dispense 20 µL into corresponding wells.*

5. *Add 5 µl of each DNA sample at 10 ng/µl, 5 µl of Haddock Positive Control and 5 µl of the Negative Controls* into the appropriate wells.*

   We recommend making each sample analysis in duplicate.

6. *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

The following instructions must be followed in order to setup the amplification program:

7500 Fast o StepOne Real-Time PCR system (Thermo Scientific)

- *Experiment type: Quantitation-Standard curve*
- *Ramp rate: standard*
- *Reaction volume: 25 µL*
- *Reference ROX™: include*
- *TaqMan® probes fluorophores:*

Probe	Reporter	Quencher
Haddock	FAM™	MGB
IPC	VIC®	MGB

***Table 3.** Probes information*

- *Optimal program:*

Fields	Step 1 Enzyme activation	Step 2 PCR	
Cycle Number	1 initial cycle	40 cycles	
		Denaturation	Primers binding / Extention
Temperature	95ºC	95ºC	60ºC
Time	10 minutes	15 seconds	1 minute*

***Table 4**. 7500 FAST or StepOne Optimal PCR program*

**Fluorescence detection*

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3. Analysis of results

Before analysing the samples results, you should stablish the threshold in 0.4 and, also, 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®.*
- ***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 Melanogrammus aeglefinus DNA (FAM™) is detected, because the PCR reagents are exhausted before amplification of the IPC begins.

Haddock

Amplification in the FAM™ channel indicates presence of Melanogrammus aeglefinus 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 Melanogrammus aeglefinus DNA.

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In samples where no amplification in the FAM™ channel is seen, we can conclude that no Melanogrammus aeglefinus DNA is detected or that their 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:

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

***Table 5.** 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:

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Controls	Haddock Master Mix		Interpretation
	Haddock	IPC	
Positive control	+	+	Expected result
	-	-	PCR Amplification Failure[1]
Extraction negative control	-	+	Expected result
	+	+	Contamination in the DNA extraction procedure[2]
PCR negative control	-	+	Expected result
	+	+	PCR contamination with Melanogrammus aeglefinus DNA[3]

Table 6. *Interpretation of possible results*

- *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 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 Melanogrammus aeglefinus 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.*