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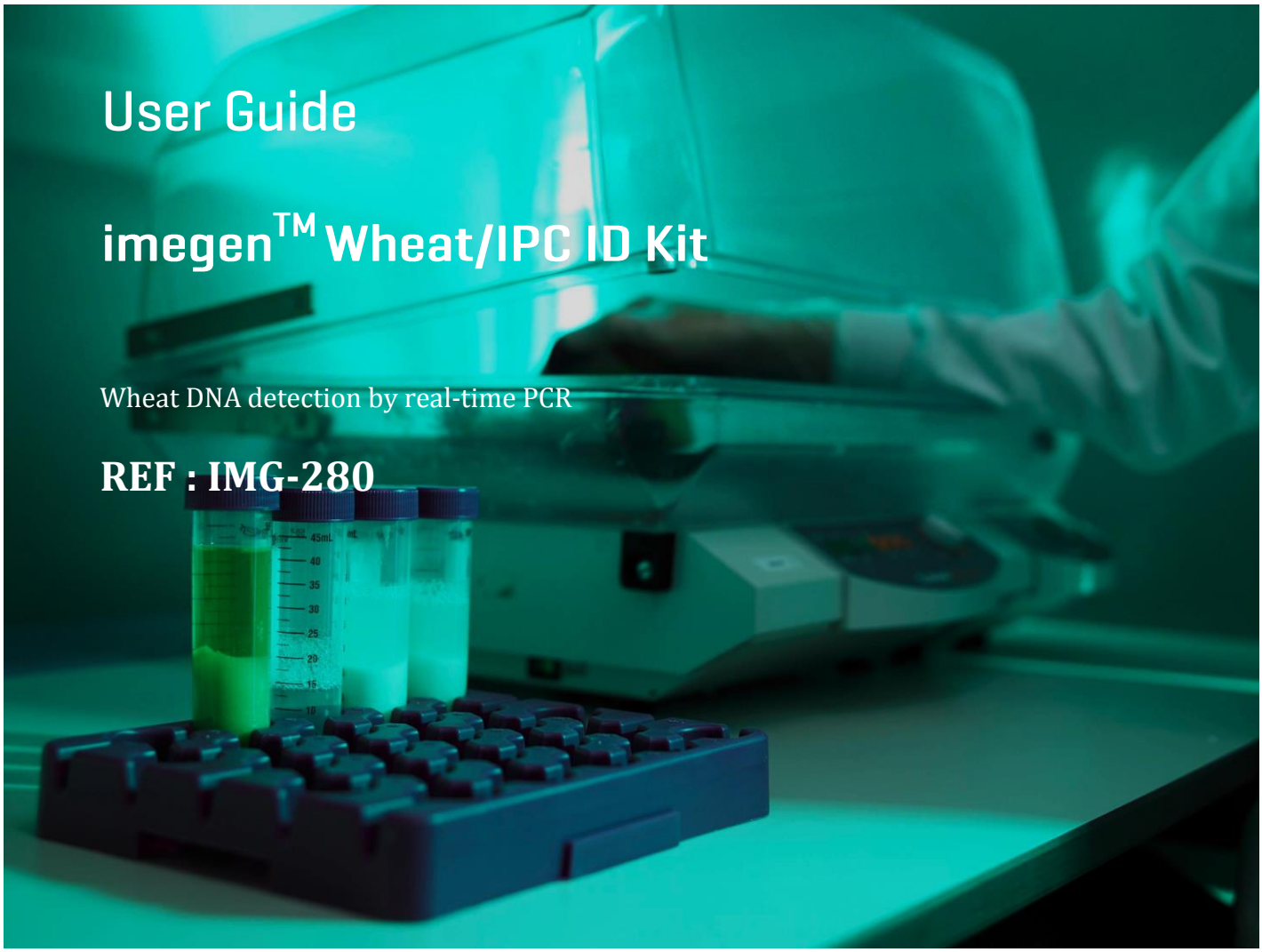
Food Safety



User Guide

imegen™ Wheat/IPC ID Kit

Wheat DNA detection by real-time PCR

REF : IMG-280

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All products sold by the imegen are subjected to rigorous quality control. The ***imegen™ WHEAT/IPC 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*
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1. Product information

Kit description

Genetically modified organisms (GMOs) are widely distributed and extensively cultivated in crops worldwide.

The European Union has established a legal framework to regulate the use, release into the environment and, above all, labelling of foodstuffs containing genetically modified organisms.

The ***imegen-Wheat/IPC ID kit****, allows the detection of wheat DNA in a sample by amplification of an endogenous wheat gene known as lipid transfer protein 1 precursor (LTP1500).*

Wheat/IPC DNA detection is done by real time multiplex PCR using two TaqMan®-MGB probes. One of them, labelled with FAM™ dye, specifically detects Wheat DNA. And the other labelled with VIC® dye, specifically detects an Internal Positive Control, which is used to rule out inhibitors in the sample and check the correct functioning of the assay.

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).*

Content and storage of the kit

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

- *Amplification primers for the PCR systems.*
- *The system contains two probes. One of them labelled with FAM™ for the specific Wheat DNA detection and other labelled with VIC® for the IPC detection.*
- *Positive control.*

The kit contents the necessary reagents to perform 48 reactions:

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

Table 1**. Kit components and storage temperature of* ***imegen-Wheat/IPC ID Kit

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Equipment and material required but not supplied

In the following table the equipment and material requirements for using ***imegen-Wheat/IPC 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

imegen-Wheat/IPC ID Kit *can detect a percentage above 0.1% of wheat DNA.*

The limit of detection in processed samples varies depending on the composition and food processing.

2. Real time PCR

PCR reactions preparation

***imegen-Wheat/IPC ID Kit** is designed to determine, in a single PCR reaction, the presence or absence of Wheat 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 Wheat 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
Wheat 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
Wheat-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	50 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 7500 fast and 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, in all the channels (FAM™ and 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 Wheat DNA (FAM™) is detected, because the PCR reagents are exhausted before amplification of the IPC begins.

WHEAT

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

In samples where no FAM™ channel amplification is seen, we can conclude that Wheat DNA is not 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 obtained result:

Wheat Master Mix		Interpretation
Wheat	IPC	
-	-	PCR Amplification Failure[1]
+	-	PCR inhibitors presence in the sample*
+	+	Wheat DNA is detected
-	+	Wheat DNA is not present in the sample

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