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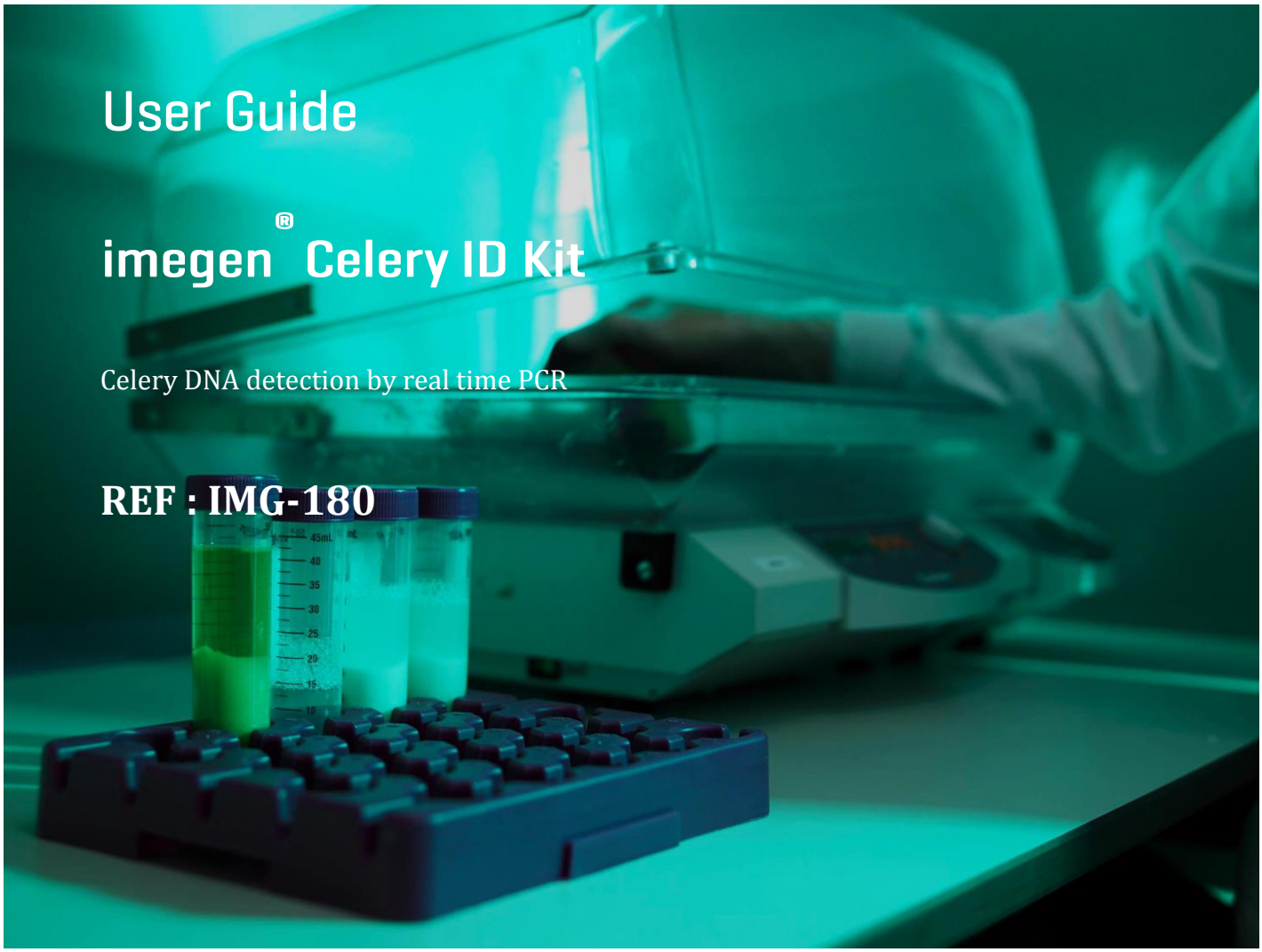

User Guide

imegen® Celery ID Kit

Celery DNA detection by real time PCR

REF : IMG-180

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All products sold by the Imegen are subjected to rigorous quality control. The ***imegen® Celery 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 preserve the health of consumers, it has been developed some kits to detect plant species and food allergens based on the latest DNA technology, used to verify the presence or absence of allergenic ingredients in any food.

Nowadays, celery is widely cultivated in temperate regions around the world, particularly in Europe and North America. And its use is becoming more common and common.

Identification of celery presence in food samples is an essential step in order to verify the origin and traceability of the used food, as well as a necessary quality control for handling and cleaning processes of production lines.

***Imegen-Celery ID Kit** allows determining the presence of DNA of celery [Apium graveolens] in any food.*

Celery DNA detection is done by real time PCR using two TaqMan®-MGB probes. One of them, labelled with FAM™ dye, specifically detects one chloroplast DNA sequence of celery. The second probe is labelled with VIC® and detects an Internal Positive Control (IPC), 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 **GMO Extraction Kit** (Part No: 4466336).*

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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 celery identification and other labelled with VIC® for the IPC detection.*
- *Celery Positive control.*

The kit contents the necessary reagents to perform 48 reactions:

Reagents	Color	Amount	Storage
Celery Master Mix	Green pad	360 µL	-20ºC
General Master Mix	White pad	600 µL	4ºC
Positive control	Green cap	60 µL	-20ºC

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

Equipment and material required but not supplied

*In the following table the equipment requirements for using **Imegen® Celery 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 allergen standard. This limit has been estimated using standard manufactured in our laboratory.

***Imegen-Celery ID Kit** can detect blends containing a percentage above 0.0001% (equivalent to 1 ppm) of celery. 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
Wheat	ND
Corn	ND
Rice	ND
Soy	ND
Rape	ND
Celery	D
Nut	ND
Peanut	ND
Almond	ND
Hazelnut	ND
Pistachio	ND
Sesame	ND
White pepper	ND
Cumin	ND
Yellow mustard	ND
Black mustard	ND
Cheakpea	ND
Macadamia Nut	ND
Lupin	ND
Zucchini	ND
Red pepper	ND
Tomato	ND
Onion	ND
Green Bean	ND
Potato	ND
Broccoli	ND

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Meat Species	Results
Carrot	ND
Cotton	ND
Barley	ND
Rye	ND
Oat	ND

Table 2. *Specificity of* ***imegen® Celery ID Kit***

D: Detected

ND: Not Detected

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2. Real time PCR

PCR reactions preparation

***Imegen®-Celery ID Kit** is designed to determine, in a single PCR reaction, the presence or absence of celery 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 Celery 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
Celery 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

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

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

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

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

***Table 4.** Probes information*

- *Optimal program:*

Fields	Step 1 Enzyme activation	Step 2 PCR	
Cycle Number	1 initial cycle	50 cycles	
		Denaturation	Primers binding/Extention
Temperature	95ºC	95ºC	60ºC
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.

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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 celery DNA (FAM™) is detected, because the PCR reagents are exhausted before amplification of the IPC begins.

Celery

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

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

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

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

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