

RapidFinder™ Fish ID Kit

SKU A24398

For testing of Food and Environmental samples only.

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1. Kit Description

Identification of animal 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.

RapidFinder™ Fish ID Kit allows determining the presence of DNA of fish in any food.

Fish DNA detection is done by real time PCR using three TaqMan®-MGB probes. Two of them, labelled with FAM™ dye, specifically detect mitochondrial DNA sequence of fish. The third 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 fish and other species. **RapidFinder™ Fish ID Kit** can detect blends containing a percentage below 0.1% (w/w) of fish. 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).

2. 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:

Species	Results	Species	Results
Sheep	ND	Deer	ND
Goat	ND	Chicken	ND
Horse	ND	Turkey	ND
Beef	ND	Duck	ND
Pork	ND	Ostrich	ND

Species	Results	Species	Results
Atlantic Salmon	D	European eel	D
Angler	D	Red bandfish	D
Cod	D	Prawn	ND
Hake	D	Norway lobster	ND
Halibut	D	Shrimp	ND
Seabass	D	Mussel	ND
Anchovy	D	Clam	ND
Sole	D	Sepia	ND
Dab	D	Squid	ND
Trout	D	Octopus	ND
Blue shark	D	Human	ND

D: Detected

ND: Not Detected

Primers and probes contained in this kit have been designed to detect any kind of fish. In food is more common finding commercial species as shown in the table above, which also have used to perform the validation report. However, it is possible to use this kit in order to detect fish, in general.

3. Kit contents and storage

The Kit contents the necessary reagents to perform 48 reactions:

Reagents	Identification	Amount	Storage
Fish Master Mix	Yellow pad	360 µl	-20°C
General Master Mix	White pad	600 µl	4°C
Positive Control	Yellow cap	60 µl	-20°C

4. Equipment requirements

In the following table the equipment requirements for using **RapidFinder™ Fish 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

5. Consumables

Needed consumables are shown in the following table:

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

6. Amplification reactions procedure

RapidFinder™ Fish ID Kit is designed to determine, in a single PCR reaction, the presence or absence of fish DNA and the internal positive control.

We recommend using, the positive control included in this kit for each run. This positive control contains 0.1% of fish DNA.

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 two Master Mix and the Positive Control vial
2. Vortex each reagent and keep cold.
3. In a 1.5 ml tube, add 7.5 µl of Fish Master Mix and 12.5 µl of General Master Mix for each reaction included in the same PCR run. Vortex and pipette 20 µl per well or tube of 0.2 ml.
4. Add 5 µl of each DNA sample at 10 ng/µl, into the appropriate wells. We recommend making each sample analysis in duplicate.
5. Add 5 µl of Positive Control and Negative Controls* into the appropriate wells.
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 submitted 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.*

7. PCR amplification program

Amplification reactions must be submitted to the following PCR program:

Temperature	Time	Cicles
95°C	10 minutes	1
95°C	15 seconds	40
60°C	1 minute	

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.

8. Analysis of results

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 FAMTM 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 fish DNA (FAMTM) is detected, because the PCR reagents are exhausted before amplification of the IPC begins.

Fish

Amplification in the FAMTM channel indicates presence of fish DNA in the sample.

It is necessary to check if sample Ct is less than the Ct_{cut-off} in order to determine if one reaction of amplification is positive. Any reaction of amplification with Ct upper than Ct_{cut-off} may be considered as negative. The Ct_{cut-off} is equal than the positive control Ct (0.1%) plus 3.32.

$$Ct_{cut-off} = 3.32 + Ct_{Positive Control}$$

Note: Any sample with a Ct equal than Ct_{cut-off} contains approximately 0.01% of fish DNA.

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

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

* 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	Master Mix Fish		INTERPRETATION
	Fish	IPC	
Positive Control	+	+	Expected result
	-	-	PCR Amplification Failure ¹
Extraction Negative Control	-	+	Expected result
	+	+	Contamination in the fish DNA extraction procedure ²
PCR Negative Control	-	+	Expected result
	+	+	PCR contamination with fish 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 fish 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 contamination with fish 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.

9. Quality control

All products manufactured and marketed by Instituto de Medicina Genómica are submitted to a rigorous quality control process. This kit has passed all the internal validation tests, thus guaranteeing the reliability and reproducibility of each assay.

The Certificate of Analysis corresponding to your kit can be consulted by entering the batch number in the Analytical Kits section on the web page www.imegen.es.

10. Customer support

For the latest services and support information for all locations, go to:
www.lifetechnologies.com/support

Food Safety support

Website: <http://www.lifetechnologies.com/foodsafety>

Support email: foodsafety@lifetech.com

Phone number in North America: 1-800-500-6855

Phone number outside of North America: Visit www.lifetechnologies.com/support, select the link for phone support, and select the appropriate country from the dropdown menu.

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