GENERON
Advanced Transfer Technologies

SPECIALfinder DETECTION ASSAY FISH

Cat. N. PAV20A

User Guide

1 - Introduction

Food allergies are an adverse immune response to a food protein that are the most common allergic compound. Food allergies are an important concern for human health; in fact, the presence of specific proteins in any food matrix can cause an allergic reaction IgE mediated. Allergic reactions may have a broad spectrum, which varies on the basis of the individual sensitivity thus generating in some cases severe anaphylactic reactions. Indeed, food correct labeling is of great importance to inform consumers about presence of any allergic substance other than achieve a high level of health protection. Unfortunately, although known allergens can be included in the product (and in the product label) by the food producer, potentially hazardous allergenic residues/contaminants can be present as result of common industrial practices. Cross-contamination between raw materials, production lines or equipment, is a common cause of unwanted allergen transfer between products intended for different scopes.

For all these reasons, developing a detection method for allergic substances ensures customers protection in accordance with food labeling regulations. The SPECIALfinder Fish Detection Assay provide the user with a simple and reliable procedure for the detection of DNA related to species potentially allergenic, in food and feed matrices as well as swabs. Such a detection is an *Indirect Proof* of the potential presence of the Fish proteins into the matrix, being proteins the real allergens.

This assay utilizes the polymerase chain reaction (PCR) to amplify a genetic target typical of the allergenic species. PCR technique can typically detect up to 1-10 copies of the target sequence but the real detection/quantification limit depends on industrial processing degree, sample matrix, DNA extraction and, lastly, on the DNA content of the sample. Genome size of the complex samples under investigation can deeply impact the Limit Of Detection (LOD) also, in addition it does exist a theoretical LOD you cannot go below, given an advised maximum load between 2 and 4 ng DNA/µl reaction mix.

Generon in-house validation: the LOD has been calculated as copy number by means of ddPCR (Droplet Digital PCR), a novel technique capable to count physically the copy number of a selected amplifiable target. With SPECIALfinder Fish an average count of 5-10 copies was obtained. DNA was extracted using Generon ION Force DNA Extractor FAST (Cat. N. EXD001).

The LOD for this assay was experimentally determined between 1 and 0.5 ppm and depends on sample matrix, processing grade, DNA preparation and DNA content.

2 - SPECIALfinder Fish Detection Assay

When used along with GENERase ULTRA PLUS Mastermix (Cat. N. ENG009) this Real-Time PCR assay detects a specific DNA sequence in the DNA of Fish in less than 1.5 hours. The amplification of the target sequence is measured by the use of a specific fluorescence-labeled probe (FAM).

2.1 - Assay Content

	Box 50 reactions		Box 100 reactions	
	N. vials	Volume (µl)	N. vials	Volume (µl)
SPECIALfinder OLIGO Mix * (OLIGOS and Probe pre-blended mix)	1	250	2	250
Positive Control	1	85	2	85
Negative Control	1	200	1	200

** reagents are supplied with a 5% of extra volume.*

We suggest to use SPECIALfinder Fish Detection Assay along with the following Polymerase Enzyme Ready-to-use mastermix: GENERase ULTRA PLUS Mastermix (Cat. N. ENG009). When using this GENERase ULTRA PLUS an additional detection channel (HEX) becomes available to detect the Internal Amplification Control (IAC) to excluding false negative results due to a PCR inhibition.

2.2 - Storage & Expiry information

Expiry date: see date on the packaging, product validity refers to the product kept intact in its original packaging. Protect reagents from light exposure as far as OLIGO Mix reagents are photosensitive. Store frozen.

3 – Materials and equipments needed

3.1 – Extraction[1]

Material/Equipment	Source
Extraction Kit	Generon ION Force DNA Extractor FAST (Cat. N. EXD001)
Chemicals: n-esane	Lab Suppliers
Tubes, 50 ml and 15 ml	Generon or other Lab Suppliers
DNAse/RNAse Free Water	Generon or other Lab Suppliers
Vortexer	Generon or other Lab Suppliers
Benchtop Centrifuge for 50 ml Tubes	Generon or other Lab Suppliers
Thermal Water Bath or Block	Generon or other Lab Suppliers
Pipette sets	Generon or other Lab Suppliers
Pipette tips (Barrier)	Generon or other Lab Suppliers
Tube rack for 1.5 ml tubes	Generon or other Lab Suppliers
2.0 and 1.5 ml micro-tubes	Generon or other Lab Suppliers
Micro centrifuge for 1.5-2.0 ml micro-tubes	Generon or other Lab Suppliers
DNA Extraction VACUUM BOX + Vacuum pump or Venturi meter	Generon or other Lab Suppliers

Each step of sample preparation (grinding, transferring, weighing, etc.) must be done according to GLP so that chance of cross-contamination between samples is minimized. It is recommended to use disposable equipment when possible.
If the food samples are not in a powdered or granular form, they should be processed (grinded or blended) before DNA extraction. The majority of DNA extraction methods supports from 20 to 50 mg of starting material. Generon ION Force DNA Extractor FAST (Cat. N. EXD001) allows processing up to 20 grams of starting material in order to maximize sample's lot representation.
Once the sample has been pulverized/homogenized, it can be weighed and the appropriate amount extracted according to DNA extraction method selected. Refer to manufacturer user manual for extraction procedure details.

3.3 – Detection via Real-Time PCR

Material/Equipment	Source
Real-Time PCR System [2]	Generon or other Lab Suppliers
Specialfinder Fish Detection Assay	Generon (Cat. N. PAV20A)
GENERase ULTRA PLUS Mastermix	Generon (Cat. N. ENG009)
Optical Adhesive Seal and Optical reaction plate or Optical Caps and Strips	Generon or other Lab Suppliers
Micropipette sets	Generon or other Lab Suppliers

(1) Equipment necessary only when ION Force DNA Extractor FAST (Cat. N. EXD001) is used.
(2) The assay can be used with Biorad CFX and MiniOpticon, Stratagene MxSeries, ABI 7300-7500-7900-StepONE-StepONE Plus, Light Cycler 480, Eppendorf realplex, Rotor-Gene Q etc. The assay is not compatible with Roche Light Cycler I and II.

4 – Real-Time PCR detection

4.1 – Reaction setup

I. Allow the reagents to thaw (GENERase ULTRA PLUS Mastermix, SPECIALfinder OLIGO MIX, Positive Control and Negative Control). Vortex tubes when thawed and spin to collect contents at the bottom of the vial.

II. Mix 250 µl of SPECIALfinder OLIGO Mix with 500 µl of GENERase ULTRA PLUS Mastermix to prepare SPECIALfinder Working Mastermix (WMX).

III. Vortex briefly and spin down in order to homogenize the mix.

IV. Transfer 15 µl of WMX into each well.

V. Add 5 µl of Negative Control into wells acting as negative control.

VI. Add 5 µl of each sample into wells testing the unknown samples.

VII. Add 5 µl of Positive Control into wells acting as positive control.

VIII. Close wells and ensure no bubbles are present at the bottom of the wells.

IX. Spin briefly optical PCR tubes or plates.

4.2 – Instrument setup

With GENERase ULTRA PLUS Mastermix set the following parameters on your thermocycler:

I. Total Reaction volume: 20 µl

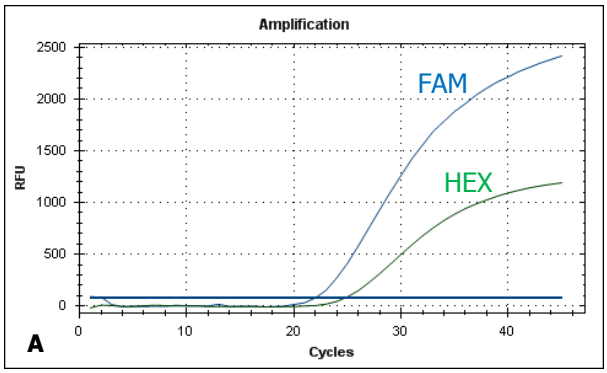
II. Fluorophores/Quenchers: Target Fish (FAM/BHQ1-NFQ); Internal Amplification Control (HEX/BHQ1-NFQ). Depending on your thermocycler, you can replace HEX detector in the plate setting with VIC or JOE in case your own Real Time Platform does not possess the HEX reading channel.

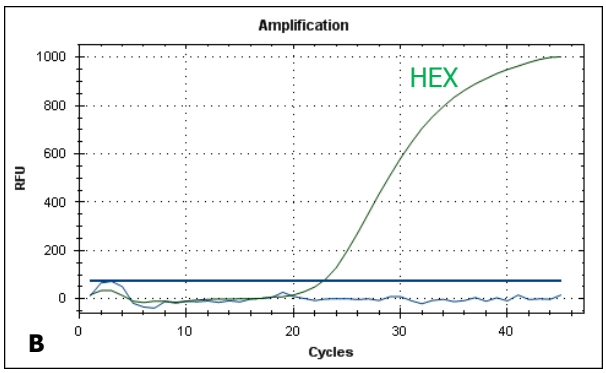
III. Thermal profile:

Step	T (°C)	Duration	Loops
Taq Activation	95	3 min	1
DNA Denaturation	95	10 sec	45
Annealing/Extension + Plate Reading	57	60 sec	

5 – Data Interpretation

Results evaluation must be done according to the analysis software recommended by the Real-Time PCR instrument manufacturer. After performing PCR, each individual sample is analyzed through the instrument software to produce a Cq value (quantification cycle) for each reporter dye. These values are used to determine the presence (Qualitative Test) of allergen into the sample. See below an example of the graphics obtained for a positive (Fig. A) and a negative (Fig. B) control, respectively for the allergen target amplification (blue line) and for the IAC amplification (green line).





After setting the baseline, the analysis outcome should be evaluated following the indications below.

If the following conditions are met:

TEST	Fish (FAM)	Internal Amplification Control (HEX)
Positive Control	+	+
Negative Control	-	+

Then the possible results for any sample are:

TEST	Fish (FAM)	Internal Amplification Control (HEX)
Positive Sample	+	+/-
Negative Sample	-	+
Invalid Sample (inhibited)	-	-

In case of inhibition DNA isolation and purification for the sample need to be improved or you may need to dilute your sample before performing a new test. Refer to the Troubleshooting paragraph (section 8) for further suggestions.

6 – Inclusivity Panel

Species tested for inclusivity				
Atlantic Cod *(Gadus morhua)*	Alaska Pollock *(Gadus chalcogrammus* (synonym: *Theragra chalcogramma*))	Anchovy *(Engraulis encrasicolus)*	Argentine Hake *(Merluccius hubbsi)*	Argentine Seabass *(Acanthistius brasilianus)*
Atlantic Bluefin Tuna *(Thunnus thynnus)*	Atlantic Mackerel *(Scomber scombrus)*	Atlantic Pomfret *(Brama brama)*	Atlantic Salmon *(Salmo salar)*	Atlantic Sturgeon *(Acipenser sturio)*
Bearded Brotula *(Brotula barbata)*	Bigeye Tuna *(Thunnus obesus)*	Big-Scale Sand-Smelt *(Atherina boyeri)*	Blackspotted *(Protonibea diacanthus)*	Blue Shark *(Prionace glauca)*
Brill *(Scophthalmus rhombus)*	Brown Meagre *(Sciaena umbra)*	Canary Drum *(Umbrina canariensis)*	Capelin *(Mallotus villosus)*	Coho Salmon *(Oncorhynchus kisutch)*
Common Sole *(Solea vulgaris)*	Deep-Water Cape Hake *(Merluccius paradoxus)*	Derbio *(Trachinotus ovatus)*	Devil Anglerfish *(Lophius vomerinus)*	Dory *(Zeus faber)*
Duskytail Grouper *(Epinephelus bleekeri)*	Eel *(Anguilla Anguilla)*	European Hake *(Merluccius merluccius)*	European Perch *(Perca fluviatilis)*	European Pilchard *(Sardina pilchardus)*
European Plaice *(Pleuronectes platessa)*	French Sole *(Solea lascaris* (synonym: *Pegusa lascaris*))	Gilthead Seabream *(Sparus aurata)*	Goatfish *(Parupeneus* spp.)	Goldstripe Sardinella *(Sardinella gibbosa)*
Greater Amberjack *(Seriola dumerili)*	Grey Mullet *(Mugil cephalus)*	Halibut *(Hippoglossus hippoglossus)*	Herring *(Clupea harengus)*	Icefish *(Neosalanx taihuensis)*
Keta Salmon *(Oncorhynchus keta)*	Lumpfish *(Cyclopterus lumpus)*	Mediterranean Horse Mackerel *(Trachurus mediterraneus)*	Megrim *(Lepidorhombus whiffiagonis)*	Narrownose Smooth-Hound *(Mustelus schmitti)*
Nile Perch *(Lates niloticus)*	North Pacific Hake *(Merluccius productus)*	Northern Pike *(Esox lucius)*	Oceanic Sole *(Synaptura* spp.)	Pacific Cod *(Gadus macrocephalus)*
Pandora *(Pagellus erythrinus)*	Patagonian Whiphake (*Macruronus novaezelandiae*)	Peruvian Anchovy (*Engraulis ringens*)	Pink Cusk-Eel *(Genypterus blacodes)*	Pink Salmon *(Oncorhynchus gorbuscha)*
Pink Snapper *(Pagrus auratus (*synonym: *Chrysophrys auratus))*	Rainbow Trout *(Oncorhynchus mykiss)*	Red Mullet *(Mullus barbatus)*	Redfish *(Sebastes marinus (*synonym: *Sebastes norvegicus))*	Rock Grenadier *(Coryphaenoides rupestris)*
Saithe *(Pollachius virens)*	Sand Steenbras *(Lithognathus mormyrus)*	Sapphirine Gurnard *(Trigla lucerna* (synonym: *Chelidonichthys lucerna*))	Seabass *(Dicentrarchus labrax)*	Senegalese Tonguesole *(Cynoglossus senegalensis)*
Shallow-Water Cape Hake *(Merluccius capensis)*	Shortfin Mako *(Isurus oxyrhincus)*	Skipjack Tuna *(Katsuwonus pelamis)*	Smooth Oreo *(Pseudocyttus maculatus)*	Smooth-Hound *(Mustelus mustelus)*
South Pacific Hake *(Merluccius gayi)*	Spanish Sardine *(Sardinella aurita)*	Spiny Dogfish *(Squalus acanthias)*	Swordfish *(Xiphias gladius)*	Thornback Ray *(Raja clavata)*
Turbot *(Psetta maxima* (synonym: *Scophthalmus maximus))*	Tusk *(Brosme brosme)*	Whiting *(Merlangius merlangus)*	Winter Skate *(Leucoraja ocellata)*	Witch Flounder *(Glyptocephalus cynoglossus)*
Withmouth Croaker *(Micropogonias furnieri)*	Yellow Goosefish *(Lophius litulon)*	Yellowfin Sole *(Limanda aspera)*	Yellowfin Tuna *(Thunnus albacares)*	

Species expected positive on the basis of *in silico* analysis				
Abramis brama	*Acanthocybium solandri*	*Acanthopagrus latus*	*Acanthurus lineatus*	*Achirus lineatus*
Acipenser baerii	*Acipenser brevirostrum*	*Acipenser dabryanus*	*Acipenser fulvescens*	*Acipenser gueldenstaedtii*
Acipenser medirostris	*Acipenser mikadoi*	*Acipenser naccarii*	*Acipenser nudiventris*	*Acipenser oxyrhynchus oxyrhynchus*
Acipenser persicus	*Acipenser ruthenus*	*Acipenser schrenckii*	*Acipenser sinensis*	*Acipenser stellatus*
Acipenser transmontanus	*Aetobatus flagellum*	*Alburnus alburnus*	*Alepocephalus bairdii*	*Allocyttus niger*
Alosa alosa	*Alosa pseudoharengus*	*Alosa sapidissima*	*Aluterus scriptus*	*Ameiurus melas*
Amphilophus citrinellus	*Amphiprion clarkii*	*Amphiprion percula*	*Amphiprion polymnus*	*Anabas testudineus*
Anarhichas minor	*Anarhichas lupus*	*Anguilla australis*	*Anguilla australis schmidti*	*Anguilla bengalensis labiata*
Anguilla bicolor bicolor	*Anguilla bicolor pacifica*	*Anguilla celebensis*	*Anguilla dieffenbachii*	*Anguilla interioris*
Anguilla japonica	*Anguilla luzonensis*	*Anguilla malgumora*	*Anguilla marmorata*	*Anguilla megastoma*
Anguilla mossambica	*Anguilla nebulosa nebulosa*	*Anguilla obscura*	*Anguilla reinhardtii*	*Anguilla rostrata*
Anodontostoma chacunda	*Anoplopoma fimbria*	*Aphia minuta*	*Arctogadus glacialis*	*Argyrosomus japonicus*
Atherina hepsetus	*Atherina presbyter*	*Auxis rochei*	*Auxis thazard*	*Bahaba taipingensis (synonym: Otolithes lini)*
Balistes capriscus	*Balistes polylepis*	*Balistes vetula*	*Barbatula nuda*	*Belonophago hutsebouti*
Beryx decadactylus	*Beryx mollis*	*Beryx splendens*	*Bodianus mesothorax*	*Bodianus rufus*
Boleophthalmus boddarti	*Boops boops*	*Botia almorhae*	*Botia histrionica*	*Botia lohachata*
Botia kubotai	*Botia rostrata*	*Botia striata*	*Branchiostegus argentatus*	*Branchiostegus japonicus*
Branchiostegus albus	*Callorhinchus callorynchus*	*Callorhinchus capensis*	*Callorhinchus milii*	*Campylomormyrus compressirostris*
Carangoides armatus	*Carangoides malabaricus*	*Caranx ignobilis*	*Caranx melampygus*	*Carassius auratus*
Carassius carassius	*Carassius cuvieri*	*Carassius gibelio*	*Catla catla*	*Centrolophus niger*
Cephalopholis argus	*Cephalopholis sexmaculata*	*Cephalopholis sonnerati*	*Channa argus*	*Channa maculata*
Channa marulius	*Chanos chanos*	*Cheimerius nufar*	*Chelidonichthys capensis*	*Chelon labrosus*
Chitala blanci	*Chitala ornata*	*Chondrostoma lemmingii*	*Cirrhinus mrigala*	*Cirrhinus molitorella*
Citharinus latus	*Clupea pallasii*	*Cobitis choii*	*Cobitis elongatoides*	*Cobitis granoei*
Cobitis lutheri	*Cobitis sinensis*	*Cobitis striata*	*Cobitis takatsuensis*	*Coilia ectenes*
Coilia grayii	*Coilia lindmani*	*Coilia mystus*	*Coilia nasus*	*Coilia reynaldi*
Colisa lalia	*Colistium nudipinnis*	*Coregonus clupeaformis*	*Coregonus lavaretus*	*Coregonus nasus*

Coreoperca whiteheadi	*Coryphaena equiselis*	*Coryphaena hippurus*	*Cottus aleuticus*	*Cottus cognatus*
Cottus gobio	*Cottus hangiongensis*	*Cottus nozawae*	*Cottus poecilopus*	*Cottus reinii*
Crossocheilus latius	*Ctenopharyngodon idella*	*Cyclocheilichthys enoplos*	*Cynoglossus abbreviatus*	*Cynoglossus bilinearis*
Cynoglossus lineolatus	*Cynoglossus puncticeps*	*Cynoglossus semilaevis*	*Cynoglossus sinicus*	*Cynoscion arenarius*
Cynoscion regalis	*Cynoscion nebulosus*	*Cyprinus carpio*	*Cyprinus carpio carpio*	*Cyprinus carpio color*
Cyprinus carpio haematopterus	*Cyprinus carpio wananensis*	*Cyprinus carpio wuyanensis*	*Cyprinus carpio xingguonensis*	*Cyttus australis*
Dasyatis akajei	*Dasyatis bennetti*	*Diagramma pictum*	*Diplodus sargus*	*Dissostichus eleginoides*
Dissostichus mawsoni	*Distichodus affinis*	*Distichodus decemmaculatus*	*Distichodus fasciolatus*	*Distichodus hypostomatus*
Distichodus noboli	*Distichodus sexfasciatus*	*Drepane africanus*	*Echelus myrus*	*Echelus uropterus*
Echeneis naucrates	*Echiichthys vipera*	*Eleginops maclovinus*	*Engraulis japonicus*	*Epinephelus amblycephalus*
Epinephelus akaara	*Epinephelus areolatus*	*Epinephelus awoara*	*Epinephelus bruneus*, (synonym: *E. moara*)	*Epinephelus clippertonensis*
Epinephelus coeruleus	*Epinephelus coioides*	*Epinephelus daemelii*	*Epinephelus epistictus*	*Epinephelus fasciatomaculosus*
Epinephelus fuscoguttatus	*Epinephelus goreensis*	*Epinephelus howlandi*	*Epinephelus lanceolatus*	*Epinephelus latifasciatus*
Epinephelus macrospilos	*Epinephelus merra*	*Epinephelus miliaris*	*Epinephelus polyphekadion*	*Epinephelus quoyanus*
Epinephelus septemfasciatus	*Epinephelus sexfasciatus*	*Epinephelus spilotoceps*	*Epinephelus stictus*	*Epinephelus striatus*
Epinephelus trimaculatus	*Epinephelus tukula*	*Epinephelus undulosus*	*Escualosa thoracata*	*Esox niger*
Esox reichertii	*Ethmalosa fimbriata*	*Etmopterus baxteri*	*Etmopterus fusus*	*Etmopterus granulosus*
Etmopterus lucifer	*Etmopterus polli*	*Etmopterus pseudosqualilolus*	*Etmopterus princeps*	*Etmopterus unicolor*
Etmopterus virens	*Etroplus maculatus*	*Eugnathichthys macroterolepis*	*Euthynnus alletteratus*	*Gadus ogac*
Gambusia affinis	*Garra flavatra*	*Garra mullya*	*Garra orientalis*	*Garra rufa*
Garra spilota	*Gasterochisma melampus*	*Gasterosteus aculeatus*	*Gasterosteus wheatlandi*	*Genypterus capensis*
Glossogobius celebius	*Glossogobius circumspectus*	*Glossogobius olivaceus*	*Glyptocephalus zachirus*,	*Gobius bucchichi*
Gobius cobitis	*Gobius cruentatus*	*Gobius niger*	*Gobius paganellus*	*Gudusia chapra*
Gymnodiptychus pachycheilus	*Harpadon mirochir*	*Harpadon nehereus*	*Heterotilapia buttifokeri*	*Heteropneustes fossilis*
Hilsa kelee	*Hippoglossoides platessoides*	*Hippoglossus stenolepis*	*Hoplias malabaricus* (synonym: *Macrodon ferox*)	*Hoplostethus japonicus*
Hucho bleekeri	*Huso dauricus*	*Huso huso*	*Hyperoglyphe japonica*	*Hyporhamphus sajori*
Ictalurus balsanus	*Ictalurus furcatus*	*Ictalurus meridionalis*	*Ictalurus pricei*	*Ictalurus punctatus*
Istiophorus albicans	*Istiophorus platypterus*	*Johnius belangerii*	*Johnius gripotus*	*Kuhlia mugil*

Labeo angra	*Labeo bata*	*Labeo calbasu*	*Labeo chrysophekadion*	*Labeo cyclorhynchus*
Labeo lineatus	*Labeo pierrei*	*Labeo rohita*	*Labeo senegalensis*	*Labiobarbus lunaris*
Labrus merula	*Labrus viridis*	*Laemonema barbatulum*	*Laemonema goodebeanorum*	*Lagocephalus inermis*
Lagocephalus spadiceus	*Larimichthys crocea*	*Larimichthys polyactis*	*Lateolabrax japonicus*	*Lates calcarifer* (synonym: *Lates calcifer*)
Lemonema longipes	*Lepidocybium flavobrunneum*	*Lepidopsetta bilineata*	*Lepomis cyanellus*	*Lepomis humilis*
Lepomis macrochirus	*Leptobotia microphthalma*	*Leptobotia rubrilabris*	*Lethrinus lentjan*	*Lethrinus obsoletus*
Leuciscus rutilus	*Leuciscus waleckii*	*Liza affinis*	*Liza aurata*	*Liza ramado*
Lophius americanus	*Lophius budegassa*	*Lophius piscatorius*	*Lota lota*	*Lutjanus argentimaculatus*
Lutjanus bengalensis	*Lutjanus johnii*	*Lutjanus kasmira*	*Lutjanus malabaricus*	*Lutjanus rivulatus*
Lutjanus russellii	*Lutjanus sebae*	*Luvarus imperialis*	*Macquaria australasica*	*Makaira indica* (synonym: *Istiompax indica*)
Makaira mazara (synonym: *M. nigricans*)	*Mastacembelus armatus*	*Mastacembelus favus*	*Mastacembelus mastacembelus*	*Melanogrammus aeglefinus*
Merluccius albidus	*Merluccius angustimanus*	*Merluccius australis*	*Merluccius senegalensis*	*Mesoborus crocodilus*
Metzia mesembrinus	*Micromesistius poutassou*	*Microphysogobio tafangensis*	*Micropterus dolomieu*	*Micropterus floridanus*
Micropterus salmoides salmoides	*Microstomatichthyoborus bashforddeani*	*Microstomus pacificus*	*Misgurnus anguillicaudatus*	*Mogurnda adspersa*
Molva molva	*Monotrete leiurus*	*Morone chrysops*	*Morone saxatilis*	*Mugil curema*
Mullus surmuletus	*Muraena augusti*	*Muraena helena*	*Muraena lentiginosa*	*Mycteroperca acutirostris*
Mycteroperca bonaci	*Mycteroperca interstitialis*	*Mycteroperca jordani*	*Mycteroperca microlepis*	*Mycteroperca olfax*
Mycteroperca prionura	*Mycteroperca rosacea*	*Mycteroperca tigris*	*Mycteroperca venenosa*	*Mystacoleucus marginatus*
Nannaethiops bleheri	*Nannaethiops unitaeniatus*	*Nemacheilus rueppelli*	*Neocyttus rhomboidalis*	*Neolebias trilineatus*
Neolebias unifasciatus	*Nematalosa nasus*	*Nibea albiflora*	*Notopterus notopterus*	*Odontesthes argentinensis*
Odontesthes bonariensis	*Odontesthes hatcheri*	*Odontesthes incisa*	*Odontesthes smitti*	*Odontobutis sinensis*
Oncorhynchus clarkii henshawi (synonym: *Salmo clarkii henshawi*)	*Oncorhynchus masou masou*	*Oncorhynchus tshawytscha*	*Oreochromis mossambicus*, (synonym: *Tilapia mossambica*)	*Oreochromis niloticus*
Osmerus eperlanus	*Osmerus mordax*	*Oxyeleotris marmorata*	*Oxyeleotris urophthalmoides*	*Padogobius nigricans*
Pagellus bogaraveo	*Pagrus auriga*	*Pagrus major*	*Pagrus pagrus*	*Pampus argenteus*
Pampus chinensis	*Pampus echinogaster*	*Pampus punctatissimus*	*Paracheirodon axelrodi*	*Paralichthys dentatus*
Paralichthys olivaceus	*Paraneetroplus synsipilus*	*Paramisgurnus dabryanus*	*Paranthias colonus*	*Paranthias furcifer*
Paraplagusia bilineata	*Paraplagusia blochii*	*Paraplagusia japonica*	*Parastromateus niger*	*Patagonothen longipes*
Pelthorhamphus novaezeelandiae	*Peprilus triacanthus*	*Perca flavescens*	*Phago boulengeri*	*Phoxinus eos*

Phoxinus oxycephalus jouyi	*Phoxinus percnurus mantschuricus*	*Phoxinus percnrus sachalinensis*	*Phycis blennoides*	*Pinjalo pinjalo*
Platichthys stellatus	*Plectropomus leopardus*	*Plesiomyzon baotingensis*	*Pleurogrammus azonus*	*Pleurogrammus monopterygius*
Pollachius pollachius	*Pomatomus saltatrix*	*Pomatoschistus knerii*	*Pomatoschistus minutus*	*Pristipomoides multidens*
Pristolepis malabarica	*Protosalanx chinensis*	*Psettodes erumei*	*Pseudobrama simoni*	*Pseudorasbora parva*
Puntius semifascicolatus	*Puntius snyderi*	*Rachycentron canadum*	*Rasbora borapetensis*	*Rastrelliger kanagurta*
Reinhardtius evermanni (synonym: Atheresthes evermanni)	*Reinhardtius hippoglossoides*	*Rexea solandri*	*Rhabdosargus globiceps*	*Rhodeus pseudosericeus*
Romanogobio tenuicorpis	*Ruvettus pretiosus*	*Salmo trutta trutta*	*Salvelinus alpinus*	*Salvelinus fontinalis*
Salvelinus leucomaenis	*Sander canadensis*	*Sarda sarda*	*Sardinella albella*	*Sardinella maderensis*
Sardinops melanostictus	*Saurida microlepis*	*Saurida undosquamis*	*Scardinius erythrophtalmus*	*Scarus forsteni*
Scarus frenatus	*Scarus ghobban*	*Scarus rubroviolaceus*	*Scarus schlegeli*	*Schedophilus velaini*
Schismatorhynchos nutka	*Schizopyge niger*	*Schizothorax dolichonema*	*Schizothorax esocinus*	*Schizothorax labiatus*
Schizothorax plagiostomus	*Schizothorax prenanti*	*Schizothorax progastus*	*Sciaenops ocellatus*	*Scomber australasicus*
Scomber colias	*Scomber japonicus*	*Scomberomorus cavalla*	*Scomberomorus commerson*	*Scomberomorus maculatus*
Scomberomorus munroi,	*Scomberomorus niphonius*	*Scomberomorus semifasciatus*	*Scophthalmus aquosus,*	*Selar crumenophthalmus*
Seriola lalandi	*Seriola quinqueradiata*	*Sicyopterus lagocephalus*	*Siganus fuscescens*	*Siganus guttatus*
Siganus puellus	*Siganus unimaculatus*	*Sillago sihama*	*Sillago vincenti*	*Solea senegalensis*
Sphyraena barracuda	*Sphyraena japonica*	*Spicara maena*	*Sprattus antipodum*	*Sprattus muelleri*
Sprattus sprattus	*Squalidus argentatus*	*Squalus megalops*	*Stizostedion lucioperca* (synonym: *Sander lucioperca*)	*Stolephorus chinensis*
Stolephorus waitei	*Symphodus cinereus*	*Symphodus melanocercus*	*Symphodus ocellatus*	*Symphodus roissali*
Symphodus tinca	*Syncrossus beauforti*	*Syncrossus berdmorei*	*Syncrossus helodes*	*Syncrossus hymenophysa*
Syncrossus reversa	*Takifugu oblongus*	*Tenualosa ilisha*	*Tetraodon nigroviridis*	*Tetrapturus albidus* (synonym: *Kajikia albida*)
Tetrapturus angustirostris	*Tetrapturus audax* (synonym: *Kajikia audax*)	*Tetrapturus belone*	*Tetrapturus georgii*	*Tetrapturus pfluegeri*
Thamnaconus tessellatus	*Theragra finmarchica*	*Thunnus alalunga*	*Thunnus maccoyii*	*Thunnus orientalis*
Thunnus tonggol	*Thymallus arcticus*	*Thymallus grubii*	*Thymallus thymallus*	*Thynnichthys polylepis*
Tinca tinca	*Trachinotus blochii*	*Trachurus japonicus*	*Trachurus trachurus*	*Trinectes maculata*
Trisopterus esmarkii	*Trisopterus minutus*	*Upeneus moluccensis*	*Variola albimarginata*	*Variola louti*
Zenopsis nebulosus				

7 – Exclusivity Panel

The following DNA extracts showed no amplification curve in a 20 µl total reaction volume:

Meat				
Beef (*Bos taurus*)	Buffalo (*Bubalus bubalis*)	Donkey (*Equus asinus*)	Duck (*Anas crecca, Cairina muschata*)	Goat (*Capra hircys*)
Goose (*Anser* spp.)	Horse (*Equus caballus*)	Poultry (*Gallus gallus domesticus*)	Quail (*Coturnix coturnix*)	Rabbit (*Oryctolagus cuniculus*)
Sheep (*Ovis aries*)	Swine (*Sus scrofa domesticus*)	Wild boar (*Sus scrofa scrofa*)	Turkey (*Meleagris gallopavo*)	
Crustaceans and Molluscs				
Clam *(Tapes semidecussatus)*	Cuttlefish *(Sepia officinalis)*	Poulpe *(Octopus cyaena)*	Squid *(Loligo edulis)*	Lobster *(Palinurus spp.)*
American Lobster *(Homarus americanus)*	Coastal Mud Shrimp *(Solenocera crassicornis)*	Kiddi Shrimp *(Parapenaeopsis stylifera)*	Lobster (*Palinurus* spp.)	Mantis Shrimp (*Squill mantis*)
Northern Prawn *(Pandalus borealis)*	Norway lobster *(Nephrops norvegicus)*	Pacific Shrimp *(Heterocarpus affinis)*	Prawn *(Penaeus vannamei*)	Red Squat Lobster (*Pleuroncodes monodon*)
Vegetabile				
Arugula (*Eruca vesicaria*)	Basil (*Ocimum basilicum*)	Bean (*Phaseolus vulgaris*)	Buckwheat (*Fagopyrum esculentum*)	Carrot (*Daucus carota*)
Eggplant (*Solanum melongena*)	Fennel (*Foeniculum vulgare*)	Garlic (*Allium sativum*)	Grape (*Vitis vinifera*)	Lemon (*Citrus limon*)
Maize *(Zea mays*)	Mango (*Mangifrea indica*)	Mushrooms (*Agaricus campestris*	Olive (*Olea europaea*)	Onion (*Allium cepa*)
Parsley (*Petroselinum crispum*)	Pepper (*Capsicum annum*)	Pine Nuts (*Pinus pinea*)	Plum (*Prunus domestica*)	Potato (*Solanum tuberosum*)
Rapeseed (*Brassica napus*)	Rice (*Oryza sativa*)	Spinach (*Spinacia oleracea*)	Sunflower (*Helianthus annuus*)	Tomato (*Solanum lycopersicon*)
		Vegetables Allergens		
Almond (*Prunus dulcis*)	Barley (*Hordeum vulgare*)	Brasilian Walnut *(Bertholletia excelsa)*	Cashew (*Anacardium occidentalis*)	Celery (*Apium graveolens*)
Durum Wheat (*Triticum durum*)	Hazelnut (*Corylus avellana*)	Kamut (*Triticum turgidum*)	Lupine (*Lupinus albus*)	Macadamia Nut (*Macadamia integrifolia*)
Mustard (*Brassica nigra*)	Oat (*Avena sativa*)	Peanut (*Arachis hypogaea*)	Pecan Nut (*Carya illinoinensis*)	Pistachio (*Pistacia vera*)
Rye (*Secale cereale*)	Sesame (*Sesamum indicum*)	Soft Wheat (*Triticum aestivum*)	Soybean (*Glycine max*)	Spelt (*Triticum monococcum*)
Walnut (*Juglans regia*)				

8 – Troubleshooting

I. Concomitant no target or IAC amplification, or amplification plots grossly abnormal. Possible causes and corrective actions:

- An excess of DNA in the target might inhibit the reaction and IAC may be affected due to an excess of DNA and/or PCR inhibitors. Test samples diluted 1:10 and 1:100. Please, use DNase/RNase Free Water to prepare dilutions.
- Inadequate sealing of optical caps/film caused sample evaporation. Redo the analysis using proper tools and proper optical caps/film to secure perfect sealing.
- Did not use the proper consumables. Redo the analysis and use only optical grade 96-well plates and optical adhesive seal or optical 8-well strips and caps.
- Samples were not properly prepared. Remake the sample DNA preps. Ensure that the DNA extraction method is properly performed.

II. Positive Control reactions failed to amplify, but other reactions appear correct (e.g. the IAC is amplified):

- Positive Control DNA was not added to the reaction wells. If other reactions look normal, there may be no need to repeat the run.

III. Negative Control reactions are positive:

- Contamination of the Negative Control vial or the SPECIALfinder PCR mix with SPECIALfinder-positive DNA. Use more care to prevent contamination while handling assay reagents and setting up assays.

In case support is needed contact Generon at: support@generon.it

9 – Disclaimers

The product is intended for research use only. Generon makes no warranty of any kind, either expressed or implied, except that the materials from which its products are made of standard quality. If any materials are defective, Generon will provide a replacement product. Generon shall not be liable for any damages, including special or consequential damage, or expense arising directly or indirectly from the use of this product. Please do not interchange components between assays of different lot numbers. This assay is designed to be used by laboratory personnel following the common molecular biology precautions.

Quick Reference Guide

Product Line:	SPECIALfinder
Part Number:	PAV20A
Type:	Qualitative
Storage:	Frozen
Execution time:	about 120 minutes
Expiry date:	see date on the packaging, product validity refers to the product kept intact in its original packaging and constantly under suitable temperature conditions as mentioned above.

Assay Box Content

	Box 50 reactions		Box 100 reactions	
	N. vials	Volume (µl)	N. vials	Volume (µl)
SPECIALfinder OLIGO Mix (OLIGOS and Probe pre-blended mix)	1	250	2	250
Positive Control	1	85	2	85
Negative Control	1	200	1	200

All reagents are supplied with a 5% of extra volume.

Not Provided Article: GENERase ULTRA PLUS Mastermix (Cat. N. ENG009) or equivalent.

Reaction Set-Up

Protect reagents from light exposure as far as OLIGO Mix reagents are photosensitive.

Before setting the analysis, we strongly advise to leave the reagents to warm up at room temperature. Vortex briefly OLIGO mix, afterwards spin to collect contents at the bottom of the vials. Spin GENERase ULTRA PLUS Mastermix before opening it.

Prepare SPECIALfinder WORKING Mastermix by adding 250 µl of SPECIALfinder OLIGO Mix into each tube prefilled with 500 µl of GENERase ULTRA PLUS Mastermix (Cat. N. ENG009) in order to obtain a single volume of 750 µl of SPECIALfinder WORKING Mastermix. Vortex briefly SPECIALfinder WORKING Mastermix with the aim of homogenizing the mix and excluding $MgCl_2$ gradient that could impair the results. Spin to collect contents at the bottom of the vial *(Note: label GENERase ULTRA PLUS vials with target name after OLIGO Mix addition)*. Vortex briefly Positive Control and samples before proceeding further, spin to collect contents at the bottom of the vial.

Transfer SPECIALfinder WORKING Mastermix and samples into the plate as follows:

Reagents per well	Volume
Unknown Sample	5 µl
Positive Control Negative Control	
SPECIALfinder WORKING Mastermix	15 µl
Final Volume	20 µl

Detector Setup

Target	Reporter Dye	Quencher Dye
Fish	FAM	BHQ1-NFQ
IAC (Internal Amplification Control)	HEX (*)	BHQ1-NFQ

(*)According to your thermocycler you can replace HEX detector in the plate setting with VIC or JOE in case your own Real Time Platform does not possess the HEX reading channel.

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Thermal cycling

Step	T (°C)	Duration	Loops
Taq Activation	95	3 min	1
DNA Denaturation	95	10 sec	45
Annealing/Extension + Plate Reading	57	60 sec	

The thermal profile presented above was optimized for GENERase ULTRA PLUS Mastermix (Cat. N. ENG009).

Results analysis

If the following conditions are met:

TEST	Fish (FAM)	Internal Amplification Control (HEX)
Positive Control	+	+
Negative Control	-	+

Then the possible results for any sample are:

TEST	Fish (FAM)	Internal Amplification Control (HEX)
Positive Sample	+	+/-
Negative Sample	-	+
Invalid Sample (Inhibited)	-	-

In case of inhibition DNA isolation and purification for the sample need to be improved or you may need to dilute your sample before performing a new test. Refer to the Troubleshooting paragraph, section 8 in the User Guide, for further suggestions.

Warning and Precaution

Please, do not interchange components of assays with different lot numbers. This assay is designed to be used by laboratory personnel following the common molecular biology precautions (GLP).

Disclaimer

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The product was internally tested by our quality control. Any responsibility is waivered if the warranty of quality control does not refer to the specific product. The user is personally responsible for data that he will obtained and/or he will supply to third parties using this assay. Once the sealed package is open the user accepts all the conditions without fail; if the package is still sealed the product can be returned and the user can be refunded.