SPECIALfinder

Real-Time PCR
Allergens Detection

GENERON
Food Safety in our Genes

In Europe, a norm concerning the control of food allergens was issued in 2003 (Dir. 2003/89/CE) and later amended in 2006 (Dir.2006/142/CE). The norm includes a list (Annex IIIa) of ingredients that must be compulsory pointed out in the label as they can elicit allergic syndromes.

Zeroing the risk of the unintentional presence of allergens can be challenging and costly

Many companies recurred to the defensive labelling using language like «…might contain traces of…». It's worth noting that defensive labeling cannot relieve from having a control plan for allergens in place. Moreover, although a specific norm is not yet in place, EU commission already pointed out that action will be taken toward the abuse of defensive labeling. This is in fact against the spirit of the norm as it might cause (i) a discrimination of allergic consumers (ii) unnecessary dietary constraints iii) undervaluation of the risks by vulnerable consumers.

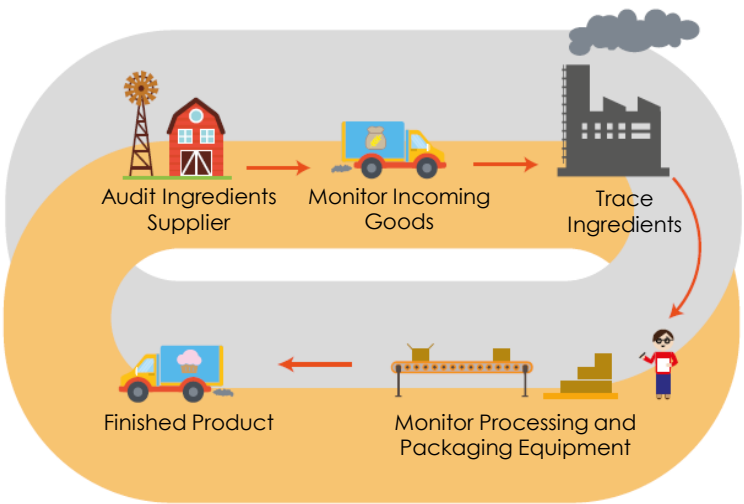
Although the dose/response thresholds have not been defined yet (as they may vary among individuals), some individuals are so sensitive to these allergens that even a minimum amount can cause serious problems or even death.

For such variability, it is impossible to base labelling rules on this parameter; nevertheless, accuracy of what stated in label is essential for a proper customer information.

Even small traces of allergenic proteins in food can provoke allergic reactions in sensitive individuals. Therefore, monitoring of allergens trace contamination in raw material and production lines as well as correct labeling of food products is becoming paramount in a globalized food market with increasing demand for quality and safety.

When a food is found contaminated by an allergen that was not declared in the label, the RASFF issue immediately an alert and the lot is recalled from the market.

The contamination of hidden allergens might occur at multiple stages of the food journey



Food companies must aim for a real and effective control of the potential allergen trace contamination in products.

Convenient and reliable methods detecting and in case quantifying allergens in order to check effectiveness of cleansing procedures and ingredients segregation protect consumers.

ELISA and PCR are the most diffused methods for the detection and quantification of food allergens

Both these methods have analytical limitations. Therefore, when possible, their combined use is the most appropriate approach according to scientific literature.

PCR is extremely useful to detect allergens in highly processed foods because DNA is less prone to degradation than proteins.

Real-Time PCR is the gold-standard for the detection of DNA of contaminating ingredients as it offers many analytical advantages, for example: the possibility to include a process control and/or a reaction control; the possibility to detect multiple allergens on the same DNA extract.

Thanks to the internal R&D activities Generon offers clients a comprehensive portfolio of products for the detection of allergens including both molecular kits and diverse process controls to increase reliability of the tests

ION Force DNA Extractor FAST

Allergens are often contaminating products in hotspots. DNA extraction kits developed by Generon provide PCR inhibitors-free DNA from large and representative samples either from vegetal or animal origin. Hence, it's possible to perform allergens detection on any kind of matrix: ingredients, semi-finished and finished products, environmental swabs.

SPECIALfinder kits

Tests developed and validated according to UNI-EN 15634-1:2009 guidelines to detect the target allergen DNA with high specificity and sensitivity. Absence of cross-reactivity was tested versus many food ingredients to avoid false positives. All the tests are made using state-of-art molecular biology grade reagents. Generon offers all the necessary pre- and post-sales support for the implementation of the methods on customers' matrices.

Reference Materials

A major challenge for allergen analysis is to obtain comparable measurement results between different laboratory measurement methods. The main requirement to ensure this comparability is the metrological traceability of all measurement results up to a common reference, which serves as an anchor or reference for scaling the data. SPECIALfinder portfolio include different reference materials serving both for method verification and process control in experimental sessions.

Instructions for ordering

SPECIALfinder DigIT Assays

0.04 µmol of primers and probe (FAM labelled) set, detecting single copy sequences according to DIN CEN/TS 15634 design.

Sets can be paired to diverse mixes and reagents for either qPCR or dPCR detection.

DAV15K-CEN SPECIALfinder DigIT Celery (acc. to CEN/TS 15634 2)

DAV02K-CEN SPECIALfinder DigIT Peanut (acc. to CEN/TS 15634 4)

DAV03K-CEN SPECIALfinder DigIT Hazelnut (acc. to CEN/TS 15634 3)

DAV01K-CEN SPECIALfinder DigIT Soy (acc. to CEN/TS 15634 5)

DAV14K-CEN SPECIALfinder DigIT Mustard (acc. to CEN/TS 15634 5)

SPECIALfinder DigiCount

DNA extracted and purified from the edible part of allergens and quantified in gc using dPCR using assays targeting genes present in single copy in allergens' genome. Each vial contains 120 µl of DNA at a concentration >1000 copies/µL.

PAV01R	SPECIALfinder DigiCount Soy
PAV02R	SPECIALfinder DigiCount Peanut
PAV03R	SPECIALfinder DigiCount Hazelnut
PAV07R	SPECIALfinder DigiCount Pistachio Nut
PAV08R	SPECIALfinder DigiCount Cashew
PAV13R	SPECIALfinder DigiCount Sesame
PAV14R	SPECIALfinder DigiCount Mustard
PAV15R	SPECIALfinder DigiCount Celery

SPECIALfinder SpyX

SpyX are laboratory reference material allowing a quantification of allergens. It's maize flour (proven allergens free), spiked with dehydrated allergens powder (from edible part). Each kit contains:

Tube 1: 5 g of maize flour allergen free
Tube 2: 5 g of maize flour allergen free spiked with 1000 ppm (w/w)

Scalar diluted extracts can be used to: monitor PCR kit performances, spike other matrices for validation purposes or to quantify contamination of unknown samples. Custom SpyX can be assembled.

PAV01X	SPECIALfinder SpyX Soy
PAV02X	SPECIALfinder SpyX Peanut
PAV03X	SPECIALfinder SpyX Hazelnut
PAV04X	SPECIALfinder SpyX Wheat
PAV05X	SPECIALfinder SpyX Pecan Nut
PAV06X	SPECIALfinder SpyX Nut
PAV07X	SPECIALfinder SpyX Pistachio
PAV08X	SPECIALfinder SpyX Cashew
PAV09X	SPECIALfinder SpyX Almond
PAV10X	SPECIALfinder SpyX Rye
PAV11X	SPECIALfinder SpyX Oats
PAV12X	SPECIALfinder SpyX Barley
PAV13X	SPECIALfinder SpyX Sesame
PAV14X	SPECIALfinder SpyX Mustard
PAV15X	SPECIALfinder SpyX Celery
PAV16X	SPECIALfinder SpyX Brazil nut
PAV18X	SPECIALfinder SpyX Lupin
PAV19X	SPECIALfinder SpyX Crustaceans
PAV20X	SPECIALfinder SpyX Fish
PAV21X	SPECIALfinder SpyX Macadamia
PAV22X	SPECIALfinder SpyX Shellfish
PAV23X	SPECIALfinder SpyX Pine nut
PAV24X	SPECIALfinder SpyX Buckwheat

DNA Extraction

EXD001 ION Force DNA extractor FAST (100 extr.)

EXD018K ION Spin K DNA Prep One-for-All (50 extr.)

EXD010 Proteinase K resuspension Buffer (1 Bottle)

EXD011 Proteinase K (100 mg/vial)

EXD020 FASTfood Universal DNA extraction (100 ml)

DNA quantification

ACC2037 QUANTUS DNA Quantification System

ACC2038 Quantus dsDNA reagent

ACC2039 Microtubes for Quantus 0,5 ml

DNA integrity Real-Time PCR Kit (50 Tests)

The quality, integrity and amount of the DNA template influence PCR results, hence the analytical results obtained. The applicability of a specific method may therefore depend on whether the analytical sample material is processed/refined or not, and on the degree of degradation of the DNA.

According to DIN CEN/TS 15634-1 the integrity of the DNA extracted from the sample can be demonstrated detecting a DNA segment present in any eucaryotic cell.

PGE06A-50 MODIfinder Universal eukaryotic reference gene

SPECIALfinder Multicopy kits (50 Tests)

Each kit detects allergens sequences present in multiple copies. The kit contains reagents for 2-plex detection of target (FAM) and internal amplification control (HEX), a positive and a negative control.

PAV01M-50 SPECIALfinder MC Soy

PAV02M-50 SPECIALfinder MC Peanut

PAV03M-50 SPECIALfinder MC Hazelnut

PAV05M-50 SPECIALfinder MC Pecan Nut

PAV06M-50 SPECIALfinder MC Walnut

PAV07M-50 SPECIALfinder MC Pistachio Nut

PAV08M-50 SPECIALfinder MC Cashew

PAV09M-50 SPECIALfinder MC Almond

PAV13M-50 SPECIALfinder MC Sesame

PAV14M-50 SPECIALfinder MC Mustard

PAV15M-50 SPECIALfinder MC Celery

PAV16M-50 SPECIALfinder MC Brazil nut

PAV18M-50 SPECIALfinder MC Lupin

PAV19A-50 SPECIALfinder MC Crustaceans

PAV20A-50 SPECIALfinder MC Fish

PAV21M-50 SPECIALfinder MC Macadamia

PAV22A-50 SPECIALfinder MC Mollusks

PAV23M-50 SPECIALfinder MC Pine

PAV26M-50 SPECIALfinder MC Tree Nuts and Peanut

SPECIALfinder Real-Time PCR kits (50 Tests)

Each kit detects a sequence present in one or few copies in target allergen. The kit contains reagents for 2-plex detection of target (FAM) and internal amplification control (HEX), a positive and a negative control.

PAV10A-50 SPECIALfinder Rye (Segale)

PAV11A-50 SPECIALfinder Oats (Avena)

PAV17A-50 SPECIALfinder Gluten (Glutine)

PAV24A-50 SPECIALfinder Buckwheat (Grano saraceno)

PAV25A-50 SPECIALfinder Barley (Orzo)

ReMaGI

Laboratory reference material ideal as process controls and reference for detection methods verification purposes. ReMaGI are prepared following ISO 17034 indications. It's a dispersive flour (proven allergens free), spiked with dehydrated allergens powder (from edible part) evenly dispersed. Homogeneity is proven extracting down to 100 mg of material and testing the signal obtained both via ELISA and PCR. Each kit contains:

Tube 0: dispersive matrix flour allergen free
Tube 1: flour spiked with allergen at "low" level
Tube 2: flour spiked with allergen at "high" level

"Low" means 2x typical LOD of ELISA kits, "High" means 4x LOD.

RMSOY_R Soy dispersed in rice flour - **Low:** 5 mg/Kg (3 x 2 g)

RMGLU_C Wheat dispersed in corn flour - **Low:** 83,3 mg/Kg (3 x 2 g)

RMGLUHT_C HT wheat dispersed in corn flour - **Low:** 100 mg/Kg (3 x 5 g)

RMMST_C Mustard dispersed in corn flour - **Low:** 5 mg/Kg (3 x 2 g)

RMMST_W Mustard dispersed in wheat flour - **Low:** 5 mg/Kg (3 x 2 g)

RMCEL_C Celery dispersed in corn flour - **Low:** 5 mg/Kg (3 x 2 g)

RMSME_C Sesame dispersed in corn flour - **Low:** 10 mg/Kg (3 x 2 g)

RMLUP_C Lupin dispersed in corn flour - **Low:** 5 mg/Kg (3 x 2 g)

RMPNT_C Peanut dispersed in corn flour **WORK IN PROGRESS**

RMHZL_C Hazelnut dispersed in corn flour **WORK IN PROGRESS**

RMPST_C Pistachio dispersed in corn flour **WORK IN PROGRESS**

RMALM_C Almond dispersed in corn flour **WORK IN PROGRESS**

RMWLN_C Walnut dispersed in corn flour **WORK IN PROGRESS**

RMBZL_C Brazil nut dispersed in corn flour **WORK IN PROGRESS**

RMPCN_C Pecan dispersed in corn flour **WORK IN PROGRESS**

RMMCD_C Macadamia dispersed in corn flour **WORK IN PROGRESS**

RMPNE_C Pine nut dispersed in corn flour **WORK IN PROGRESS**

RMCHW_C Cashew dispersed in corn flour **WORK IN PROGRESS**

RMFSH_C Salmon dispersed in corn flour **WORK IN PROGRESS**

RMMSK_C Squid dispersed in corn flour **WORK IN PROGRESS**

RMCRS_C Shrimp dispersed in corn flour **WORK IN PROGRESS**

GENERON

INGREDIENTS AUTHENTICITY | CHEMICAL RESIDUES
MICROBIOLOGY | MYCOTOXINS
ALLERGENS | GMO

Generon S.p.A.
Via San Geminiano, 4 – 41030 San Prospero (MO) - ☎ +39 059 8637161
marketing@generon.it www.generon.it

Rev.1_250322