



SureFood® Allergen kits

Qualitative and quantitative real-time PCR analysis

- 4plex Allergen test kits
- Quantification
- Qualitative detection
- Labeling obligation



Allergen analysis with real-time PCR

Food allergies are specific food intolerances that can cause an immunological reaction due to proteins or substances. This can even lead to an anaphylactic shock which can prove fatal in some cases.

Trigger for an allergic reaction are not nucleotides (DNA), but primarily allergenic proteins. In accordance to EU Directive 2007/68/EC, the food **ingredient** needs to be declared instead of the allergenic protein or fragment on the food product for consumer protection.

Thus, different analytical methods can be used. With the detection of a listed ingredient, the food product needs to be labelled. Exceptionally, this is not necessary if the ingredient is largely removed during the production process (e.g. distillates or extracts).

Labelling obligation:

1. Cereals containing gluten (i.e. wheat, rye, barley, oats, spelt, kamut) and products thereof*
2. Crustaceans and products thereof*
3. Eggs and products thereof*
4. Fish and products thereof*
5. Peanuts and products thereof*
6. Soya beans and products thereof*
7. Milk and products thereof (including lactose)*
8. Nuts, i.e. almond, hazelnuts, walnuts, cashews, pecan nuts, Brazil nuts, pistachio nuts, macadamia nuts and Queensland nuts and products thereof*
9. Celery and products thereof*
10. Mustard and products thereof *
11. Sesame seeds and products thereof *
12. Sulphur dioxide and sulphites at concentrations of more than 10 mg/kg or 10 mg/litre expressed as SO₂
13. Lupin and products thereof *
14. Molluscs and products thereof *

Reference:

Appendix 3 of EU Directive 2007/68/EC
Appendix 2 of EU Directive 2011/1169/EC

* Exception: certain matrices

In other countries, however, different regulations apply such as the so called “big eight” in the USA and in further countries to be labeled parameters in accordance to FALCPA: Crustaceans, eggs, milk, peanuts, tree nuts, wheat soya, and sulfur dioxide.

Eggs and milk cannot be analysed with the real-time PCR, but immunological methods (ELISA, dip

stick) can be used for that. Barely suitable or even unsuitable for the PCR analysis are highly processed and/or purified products as no more or very little DNA is present, e.g. vegetable oil, gelatin, lecithin or starch.

Sulfur dioxide can be detected enzymatically, for example.



SureFood® ALLERGEN Celery,
Art. No. S3605



SureFood® ALLERGEN 4plex Macadamia/Brazil Nut/
Pecan + IAC, Art. Nr. S3403

Qualitative and quantitative analysis

The second generation of allergen kits includes an internal amplification control (IAC; FAM/HEX), and may be used for qualitative and quantitative (using external standards) detection.

DNA preparation – PREP Advanced

100 mg of a substance to be tested is lysed in 580 µL lysis buffer and 20 µl of proteinase K at 65 °C for one hour. After centrifugation and filtration via a spin filter, the DNA is bound to a spin filter, washed several times with wash buffer and eluted with 50 µL of elution buffer.

Qualitative analysis

In a two-step thermal profile (e.g. block cycler: 1 min 95 °C, 15 sec 95 °C, 30 sec 60 °C), the DNA is amplified for 45 cycles. A positive result for a qualitative test shows an exponential curve and a Ct value.

Quantitative analysis

For a risk assessment wanted by industrial producers, shown in the standard form in VITAL 2.0 concept (Voluntary Incidental Trace Allergen Labelling), quantitative values are required. Reference material SureFood® QUANTARD Allergen 40 is extracted parallel to the food sample.

Subsequently, in addition to the samples of unknown concentration, SureFood® QUANTARD Allergen 40 and a dilution series of a standard DNA are analysed by real-time PCR.

The calibrators SureFood® QUANTARD Allergen 40 consists of corn flour powder.

40 mg/kg (ppm) of the most important allergens were homogenized. Thus, it can be used as a universal calibration substance. Alternatively, the user can create matrix-specific calibration material.

The evaluation is performed with a pre-made Excel spreadsheet.

Fig. 1: SureFood® ALLERGEN QUANT data interpretation sheet

Hinweise Instructions	
Bitte entsprechende Werte in blaue Felder eintragen	
Please fill in respective values in blue cells	
Entnehmen Sie die Ergebnisse den grünen Feldern	
Please read results in green cells	
Optionale Information	Optionale Daten
Optional information	Optional data

Datum Date:			
Bearbeiter (Kürzel) Operator (code):			
Parameter / Bestellnr. Parameter / product code:			
Lot-nr. Lot no.:			
Verfallsdatum Expiry date:			
PCR Gerät PCR device:			
Steigung Slope:			
Achsenabschnitt axis intercept:			
Konzentration Referenz* [ppm**] Concentration reference sample* [ppm**]:			
Ct Referenz 1* Ct Reference 1*:			
Ct Referenz 2* Ct Reference 2*:			
Ø (Mittelwert) Ct Referenz* Ø (mean) Ct Reference*:			
Probenr. Sample no.	Probenbezeichnung sample identification	Ct Probe Ct sample	Konzentration Probe [ppm**] concentration sample [ppm**]
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			

Table 1: Composition of SureFood® QUANTARD Allergen 40 adapted to EU Directive 2007/68/EC

Allergen	Food	Allergen	Food
Gluten containing cereals	Wheat flour type 405	Nuts	Hazelnuts, almonds, walnuts, cashew nuts, pecan nuts, brazil nuts, pistachio, macadamia nuts
Crustaceans	Black tiger shrimps	Celery	Celery seeds
Egg	Egg powder (hen)	Mustard	Mustards seeds, yellow
Fish	Salmon	Sesame	Sesame seeds
Peanut	Peanuts	Lupin	Lupin seeds
Soy	Soybeans		
Milk	Milk powder (cow)		

Comprehensive analysis with 4plex screening

Rapid comprehensive screenings are provided by a 4plex system. In one single measurement, even three parameters can be analysed simultaneously. An included Internal Amplification Control (IAC) provides an inhibition control for each sample without any additional effort.

SureFood® ALLERGEN 4plex

Channel	S3401	S3402	S3403
FAM	Mustard	Peanut	Macadamia
Cy5	Soya	Hazelnut	Brazil nut
ROX	Celery	Walnut	Pecan
VIC/HEX	IAC	IAC	IAC

SureFood® ALLERGEN kits

Product	Description	No. of test/amount	Art. No.
SureFood® PREP			
Advanced	For highly processed matrices (food and feed)	50 preparations	S1053
Extraction control detection kit			
SureFast® Animal+Plant Control 3plex	Extraction control for plant or animal matrix including internal control DNA (ICD)	100 reactions	S4053
SureFood® ALLERGEN – qualitative and/or quantitative real-time PCR			
Gluten	LOD ≤ 0.4 mg/kg (ppm)	100 reactions**	S3606
Soya	LOD ≤ 0.4 mg/kg (ppm)	100 reactions**	S3601
Almond	LOD ≤ 0.4 mg/kg (ppm)	100 reactions**	S3604
Brazil nut	LOD ≤ 0.4 mg/kg (ppm)	100 reactions**	S3617
Cashew	LOD ≤ 0.4 mg/kg (ppm)	100 reactions**	S3615
Hazelnut	LOD ≤ 0.4 mg/kg (ppm)	100 reactions**	S3602
Macadamia	LOD ≤ 0.4 mg/kg (ppm)	100 reactions**	S3616
Peanut	LOD ≤ 0.4 mg/kg (ppm)	100 reactions**	S3603
Pecan	LOD ≤ 0.4 mg/kg (ppm)	100 reactions**	S3618
Pistachio	LOD ≤ 0.4 mg/kg (ppm)	100 reactions**	S3614
Walnut	LOD ≤ 0.4 mg/kg (ppm)	100 reactions**	S3607
Sesame	LOD ≤ 0.4 mg/kg (ppm)	100 reactions**	S3608
Crustaceans	LOD ≤ 0.4 mg/kg (ppm)	100 reactions**	S3612
Fish	LOD ≤ 1.0 mg/kg (ppm)	100 reactions**	S3610
Molluscs*	LOD ≤ 0.4 mg/kg (ppm)	100 reactions	S3613
Celery	LOD ≤ 0.4 mg/kg (ppm)	100 reactions**	S3605
Lupin	LOD ≤ 0.4 mg/kg (ppm)	100 reactions**	S3611
Mustard	LOD ≤ 0.4 mg/kg (ppm)	100 reactions**	S3609
SureFood® ALLERGEN – qualitative real-time PCR			
4plex Peanut/Hazelnut/Walnut + IAC	LOD ≤ 1 mg/kg (ppm)	100 reactions	S3402
4plex Soya/Celery/Mustard + IAC	LOD ≤ 0.4 mg/kg (ppm)	100 reactions	S3401
4plex Macadamia/Brazil nut/Pecan + IAC	LOD ≤ 0.4 mg/kg (ppm)	100 reactions	S3403
Buckwheat	LOD ≤ 0.4 mg/kg (ppm)	100 reactions	S7005
Oat	LOD ≤ 1 mg/kg (ppm)	100 reactions	S7004
4plex Cereals (wheat/barley/rye)	LOD ≤ 1 mg/kg (ppm)	100 reactions	S7006
Laboratory reference material for quantification			
SureFood® QUANTARD Allergen 40	Corn flour contains 12 potential allergens in food except sulphite and lactose with concentration of 40 mg/kg. The material has been developed for PCR quantification of allergens in food.	2 grams	S3301
SureFood® – qualitative real-time PCR			
Apricot	LOD ≤ 5 DNA copies	100 reactions	S7007
Rice	LOD ≤ 5 DNA copies/0.01 %	100 reactions	S6103



* Qualitative only.

** SureFood® QUANTARD Allergen 40 must be used for quantification.