



## User's Manual

# Hazelnut ELISA Kit



DEIA293



96T



This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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## PRODUCT INFORMATION

### Intended Use

Enzyme Immunoassay for the Quantitative Determination of Hazelnut in Food

### General Description

Hazelnut (*Corylus avellana*) belongs to the birch plants. With 13 % the fraction of proteins in hazelnuts is high. Many of these proteins are known for being allergenic, such as Cor a 9 and Cor a 11. Most of them are very heat resistant making them stable to different production processes. For this reason hazelnut represents an important food allergen. For hazelnut allergic persons hidden hazelnut allergens in food are a critical problem. Already very low amounts of hazelnuts can cause allergic reactions, which may lead to anaphylactic shock in severe cases. Because of this, hazelnut allergic persons must strictly avoid the consumption of hazelnuts or hazelnut containing food. Crosscontamination, mostly in consequence of the production process is often noticed. The chocolate production process is a representative example. This explains why in many cases the existence of hazelnut residues in foods cannot be excluded. For this reason sensitive detection systems for hazelnut residues in foodstuffs are required. The Hazelnut ELISA represents a highly sensitive detection system and is particularly capable of the quantification of hazelnut residues in cookies, cereals, ice cream and chocolate.

### Principles of Testing

The Hazelnut quantitative test is based on the principle of the enzyme linked immunosorbent assay. An antibody directed against hazelnut proteins is bound on the surface of a microtiter plate. Hazelnut containing samples or standards are given into the wells of the microtiter plate. After 20 minutes incubation at room temperature, the wells are washed with diluted washing solution to remove unbound material. A peroxidase conjugated second antibody directed against hazelnut proteins is given into the wells and after 20 minutes of incubation the plate is washed again. A substrate solution is added and incubated for 20 minutes, resulting in the development of a blue colour. The colour development is inhibited by the addition of a stop solution, and the colour turns yellow. The yellow colour is measured photometrically at 450 nm. The concentration of hazelnut is directly proportional to the colour intensity of the test sample.

### Reagents And Materials Provided

The kit contains reagents for 96 determinations. They have to be stored at 2-8°C. Expiry data are found on the labels of the bottles and the outer package.

1. Microtiter plate consisting of 12 strips with 8 breakable wells each, coated with anti-hazelnut antibodies.
2. Hazelnut Standards (0; 1; 4; 10; 40 ppm of hazelnut): 5 vials with 1.0 mL each, dyed red, ready-to-use.
3. Conjugate (anti-hazelnut-peroxidase): 15 mL, dyed red, ready-to-use.
4. Substrate Solution (TMB): 15 mL, ready-to-use.
5. Stop Solution (0.5 M H<sub>2</sub>SO<sub>4</sub>): 15 mL, ready-touse.
6. Extraction and sample dilution buffer (Tris): 2 × 120 mL as 10x concentrate, dyed red. Dilute 1+9 with

distilled water. Stored at 4°C the diluted buffer is stable for at least one week. If during the cold storage crystals precipitate, the concentrate should be warmed up to 37°C for 15 minutes.

7. Washing Solution (PBS + Tween 20): 60 mL as 10× concentrate. Dilute 1+9 with distilled water. Stored at 4°C the diluted buffer is stable for at least 4 weeks. If during the cold storage crystals precipitate, the concentrate should be warmed up to 37°C for 15 minutes.
8. Plastic bag to store unused microtiter strips.
9. Instruction Manual.

## Materials Required But Not Supplied

### Instrumentation

1. 100 - 1000 µL micropipets
2. Volumetric flask
3. Analytical balance
4. Mortar, mixer
5. Water bath
6. Centrifuge
7. ELISA reader (450 nm)

### Reagents

double distilled water

## Storage

Stored at 2-8°C. Expiry data are found on the labels of the bottles and the outer package. For more detailed information, please download the following document on our website.

## Assay Procedure

### 1. Reagent And Sample Preparation

Due to high risk of cross-contamination all applied instruments like applicator, mortar, glass vials etc. have to be cleaned thoroughly before and after each sample. Hazelnut proteins adhere very strongly to different surfaces. In certain cases they can resist a common dishwasher cleaning. To identify possible cross-contamination caused by previous extractions it is strongly recommended to note the sequence of the extractions.

The following sample preparation should be applied for solid samples:

- 1) To maximize homogeneity and representativeness of the sample drawing, a minimum of 5 g sample should be pulverized finely in a mortar, impact mill etc.
- 2) 1 g of the homogenized mixture is suspended in 20 mL of pre-diluted extraction buffer. Afterwards the suspension is incubated for 15 min in a preheated water bath at 60°C. To ensure good homogeneity, the samples should be shaken every two minutes.
- 3) The samples are centrifuged for 10 minutes at 2000 g. If it is not possible to separate the supernatant from

the precipitate completely, the suspension should be filtrated if necessary.

- 4) 100 µL of particle-free solution are applied per well. If the results of a sample are out of the measuring range, further dilution with the prediluted extraction and sample dilution buffer is necessary. The additional dilution has to be considered when calculating the concentration.

## 2. Assay Steps

The washing solution is supplied as 10x concentrate and has to be diluted 1+9 with double distilled water before use. In any case the ready-to-use standards provided should be determined twofold. When samples in great quantities are determined, the standards should be pipetted once before the samples and once after the samples. For final interpretation the arithmetic mean is used for calculation. In consideration of GLP and quality control requirements a duplicate measurement of samples is recommended. The procedure is according to the following scheme:

- 1) Prepare samples as described above.
- 2) Pipet 100 µL ready-to-use standards or prepared samples in duplicate into the appropriate wells of the microtiter plate.
- 3) Incubate for 20 minutes at room temperature.
- 4) Wash the plate three times as follows: Discard the contents of the wells (dump or aspirate). Pipet 300 µL of diluted washing solution into each well. After the third repetition empty the wells again and remove residual liquid by striking the plate against a paper towel. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbencies.
- 5) Pipet 100 µL of conjugate (anti-hazelnut-peroxidase) into each well.
- 6) Incubate for 20 minutes at room temperature.
- 7) Wash the plate as outlined in 4.
- 8) Pipet 100 µL of substrate solution into each well.
- 9) Allow the reaction to develop in the dark (e.g. cupboard or drawer; the chromogen is light-sensitive) for 20 minutes at room temperature.
- 10) Stop enzyme reaction by adding 100 µL of stop solution (0.5 M H<sub>2</sub>SO<sub>4</sub>) into each well. The blue colour will turn yellow upon addition.
- 11) After thorough mixing, measure absorbance at 450 nm (reference wavelength 620 nm), using an ELISA reader. The colour is stable for 30 minutes.

## Calculation

### 1. Evaluation

The following table contains an example for a typical standard curve. The binding is calculated as percent of the absorption of the 40 ppm standard. These values are only an example and should not be used instead of the standard curve which has to be measured in every new test.

Hazelnut (ppm)	% binding of 40 ppm
40	100
10	41
4	23
1	9
0	5

## 2. Calculation

The ready-to-use standards are prepared for a direct determination of sample concentrations. The dilution of samples in the extraction process as described in the above stated sample preparation procedure is already considered. Additional dilution due to high sample concentration has to be accounted for.

- 1) Calculate the average optical density (OD 450 nm) for each set of reference standards or samples.
- 2) Construct a standard curve by plotting the mean optical density obtained for each reference standard against its concentration in ppm on semi-log graph paper with the optical density on the vertical (y) axis and the concentration on the horizontal (x) axis. Alternatively the evaluation can be carried out by software. In this case the 4-parameter method should be preferred.
- 3) Using the mean optical density value for each sample, determine the corresponding concentration of hazelnut in ppm from the standard curve. Depending on experience and/ or the availability of computer capability, other methods of data reduction may be employed.

## Precision

Intra-assay Precision: 7 – 12%

Inter-assay Precision: 3 – 13%

## Sensitivity

The limit of detection (LOD) of the Hazelnut test is 0.3 ppm. The limit of quantification (LOQ) of the Hazelnut test is 1 ppm. Due to the variety of sample matrices and their influence on the blank, results less than the LOQ should be treated as negative.

## Specificity

For the following foods no cross-reactivity could be detected:

Wheat, Soy, Brazil nut, Barley, Poppy seed, Pistachio, Rye, Sunflower seed, Macadamia nut, Oats, Pumpkin seed, Chestnut, Buckwheat, Pine nuts, Cocoa, Corn, Cashew nut, Dried milk, Rice, Sesame, Gluten, Pea, Peanut, Lecithin, Chickpea, Walnut, Gelatin, Bean, Coconut, Apple, Almond.

## Linearity

The serial dilution of spiked samples (cookies, cereals, ice cream and chocolate) resulted in a dilution linearity of 87% - 121%.

## Recovery

Mean recovery was determined by spiking samples with different amounts of hazelnut:

Cookies 101%

Cereals 99%

Ice cream 90%

Chocolate 83%

## References

1. Wigotzki M, et al. (2000) – Influence of varieties, storage and heat treatment on IgE-binding proteins in hazelnuts (*Corylus avellana*). Food and Agric Immunol, 12:217-29
2. Le TM, et al. (2008) – Reported food allergy to peanut, tree nuts and fruit: comparison of clinical manifestations, prescription of medication and impact on daily life. Allergy, 63(7):910-6
3. Luettkopf D, et al. (2001) – Comparison of four variants of major allergen in hazelnut (*Corylus avellana*) Cor a 1.04 with the major hazel pollen allergen Cor a 1.01. Mol Immunol, 38:515-25
4. Ballmer-Weber BK, et al. (2008) – Predictive value of the sulfidoleukotriene release assay in oral allergy syndrome to celery, hazelnut, and carrot. J Investig Allergol Clin Immunol, 18(2):93-99