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User's Manual

Almond ELISA Kit

REF **DEIA287**

Σ **96T**

RUO

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 Almond in Food.

General Description

Almond (Prunus dulcis) belongs to the rosaceae. With 25 % the fraction of proteins in almond is high. Some of these proteins are known for being allergenic. In addition to profilin which is known to be cross-reactive to grass pollen, the almond major protein AMP has the greatest relevancy. AMP is known to be heat resistant making it stable to different production processes. For this reason almond represents an important food allergen. For almond allergic persons hidden almond allergens in food are a critical problem. Already very low amounts of almond can cause allergic reactions, which may lead to anaphylactic shock in severe cases. Because of this, almond allergic persons must strictly avoid the consumption of almonds or almond 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 almond residues in foods cannot be excluded. For this reason sensitive detection systems for almond residues in foodstuffs are required. The Almond ELISA represents a highly sensitive detection system and is particularly capable of the quantifiction of almond residues in cookies, cereals, ice cream and chocolate.

Principles of Testing

The Almond quantitative test is based on the principle of the enzyme linked immunosorbent assay. An antibody directed against almond proteins is bound on the surface of a microtiter plate. Almond 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 almond 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 almond is directly proportional to the colour intensity of the test sample.

Reagents And Materials Provided

1. Microtiter plate consisting of 12 strips with 8 breakable wells each, coated with anti-almond antibodies.
2. Almond Standards (0; 0.4; 1; 4; 10 ppm of almond): 5 vials with 1.0 mL each, dyed red, ready-to-use.
3. Conjugate (anti-almond-peroxidase): 15 mL, dyed red, ready-to-use.
4. Substrate Solution (TMB): 15 mL, ready-to-use.
5. Stop Solution (0.5 M H_2SO_4): 15 mL, ready-touse.
6. Extraction and sample dilution buffer (Tris): 2 × 120 mL as 10× 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 10x 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 a high risk of cross-contamination all applied instruments like applicator, mortar, glass vials etc. have to be cleaned thoroughly before and after each sample. 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 10× 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 de termined 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 measure ment 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-almond-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_2SO_4) 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 10 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.

Almond (ppm)	% binding of 10 ppm
10	100
4	54
1	21
0.4	13
0	6

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 almond 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: 3 - 9%

Inter-assay Precision: 3 – 13%

Inter-lot Precision: 2 - 8%

Sensitivity

The limit of detection (LOD) of the Almond test is 0.2 ppm. The limit of quantification (LOQ) of the Almond test is 0.4 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:

Milk, Sunflower seeds, Walnut, Egg, Poppy seed, Pistachio, Wheat, Sesame, Chestnut, Barley, Pine nuts, Lecithin, Rye, Cashew nut, Peach, Oats, Peanut, Plum, Rice, Hazelnut, Apricot, Corn, Pecan, Cherry, Buckwheat, Brazil nut, Cocoa, Soy, Coconut.

Linearity

The serial dilution of spiked samples (cookies, cereals, ice cream and chocolate) resulted in a dilution linearity of 85% - 98%.

Recovery

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

Cookies 91%

Cereals 107%

Ice cream 79%

Dark chocolate 69%

References

1. Roux KH, et al. (2001) – Detection and stability of the major almond allergen in foods. J Agric Food Chem, 49(5):2131-6
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3. Ben Rejeb S, et al. (2005) – Multi-allergen screening im munoassay for the detection of protein markers of peanut and four tree nuts in chocolate. Food Addit Contam, 22(8):709-15