Version 19-04/24

User's Manual

Sesame ELISA Kit

REF **DEIA297**

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 Sesame in Food.

General Description

Sesame belongs to the family of Pedaliaceae. With about 16 - 32% the fraction of proteins in sesame seed is very high. Some of these proteins, like the albumins Ses i 1 and Ses i 2 or the globulin Ses i 3 are known for being allergenic. Because of its widespread application possibilities, sesame is used in many food preparations. For sesame-allergic persons hidden sesame allergens in food are a critical problem. Already very low amounts of sesame can cause allergic reactions, which may lead to anaphylactic shock in severe cases. Because of this, sesame-allergic persons must strictly avoid the consumption of sesame containing food. Cross-contamination, mostly in consequence of the production process, is often noticed. This explains why in many cases the existence of sesame residues in food cannot be excluded. For this reason sensitive detection systems for sesame residues in foodstuffs are required. The Sesame ELISA represents a highly sensitive detection system for sesame and is particularly capable of the quantification of residues in bakery products, soups, sausage, sauces and ice cream.

Principles of Testing

The Sesame quantitative test is based on the principle of the enzyme linked immunosorbent assay. An antibody directed against sesame proteins is bound on the surface of a microtiter plate. Sesame 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 sesame 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 sesame 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. Sesame Standards (0; 2; 5; 15; 30 ppm of sesame): 5 vials with 1.0 mL each, dyed red, ready-to-use.
3. Conjugate (anti-sesame-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 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. Sesame proteins could adhere to different surfaces. 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 and sample dilution 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 theresults 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.

The following sample preparation should be applied for liquid samples:

1) 1 mL of liquid sample is diluted in 19 mL of prediluted extraction and sample dilution 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. The process is continued at point 3 of solid sample extraction process.

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 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 con tents 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 criti cal. Insufficient washing will result in poor precision and falsely elevated absorbencies.
5) Pipet 100 µL of conjugate (anti-sesame-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 30 ppm standard. These values are only an example and should not be used instead of the standard curve which has to be measured in each new test.

Sesame (ppm)	% binding of 30 ppm
30	100
15	91
5	68
2	41
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 peanut 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: 5 – 12%

Inter-assay Precision: 4 – 10%

Sensitivity

The limit of detection (LOD) of the Immunolab Sesame test is 0.2 ppm for the standard curve. Validation experiments with common matrices resulted in the following LODs [ppm].

Soup 0.2

Ice 0.2

Sausage 0.2

Salad sauce 0.2

Cracker 0.2

The limit of quantification (LOQ) of the Immunolab Sesame test is 2 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, Pumpkin seed, Brazil nut, Barley, Pine seed, Coconut, Rye, Poppy seed, Walnut, Milk, Hazelnut,

Chickpea, Egg, Peanut, Lecithin (soy), Cocoa, Cashew, Pea, Rice, Macadamia, Lupin, Corn, Pistachio, Potato, Buckwheat, Chestnut, Kiwi, Soy, Almond, Sunflower seed, Pecan.

The following cross reactions were determined:

Oats 0.0003%

Bean 0.0003%

Linearity

The serial dilution of spiked samples (sausage, cracker, salad sauce, soup, ice) resulted in a dilution linearity of 73–129%.

Recovery

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

Soup 100%

Ice 85%

Sausage 92%

Salad sauce 93%

Cracker 109%

References

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