Almond ELISA Kit

Enzyme Immunoassay for the quantitative determination of Almond in food

Catalog number: ARG80788

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INTRODUCTION

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. Cross contamination, 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 cap-able of the quantification of almond residues in cookies, cereals, ice cream and chocolate.

PRINCIPLE OF THE ASSAY

This is an Enzyme Immunoassay for the quantitative determination of Almond in food

.This assay employs the sandwich quantitative enzyme immunoassay technique. An antibody specific for Almond proteins is bound onto a pre-coated 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 HRP-conjugated secondary antibody directed against tropomyosin 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 color. The color development is inhibited by the addition of a stop solution, and the color turns yellow. The absorbance is proportional to the concentration of Almond. A standard curve is constructed by plotting absorbance values against concentrations of standards, and concentrations of unknown samples are determined using this standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	12 strips x 8-well	4°C
HRP-antibody Conjugate	15 ml (ready to use)	4°C
Standards	5 X 4 ml (ready to use) (0,0.4,1,4,10 ppm)	4°C
10x Extraction & Sample dilution buffer	2 X 120 ml	4°C
10x Wash Buffer	60 ml	4°C
TMB substrate	15 ml (ready to use)	4°C (Protect from light)
STOP solution	15 ml (ready to use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm (optional reference absorbance at 620 nm).
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- If crystals are observed in the 10X Wash buffer, Extraction Buffer and Sample dilution buffer, warm to RT or 37°C (not more than 50°C) for 15 minutes or until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- Samples contain azide cannot be assayed.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 10X wash buffer into distilled water to yield 1X wash buffer.
- 1X Extraction and Sample Dilution Buffer: Dilute 10X Extraction buffer into distilled water to yield 1X Extraction buffer.

SAMPLE COLLECTION & STORAGE INFORMATION

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. Tropomyosin 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 kind of 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 the results of a sample are out of the measuring range, further dilution with the pre-diluted extraction and sample dilution buffer is necessary. The additional dilution has to be considered when calculating the concentration.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

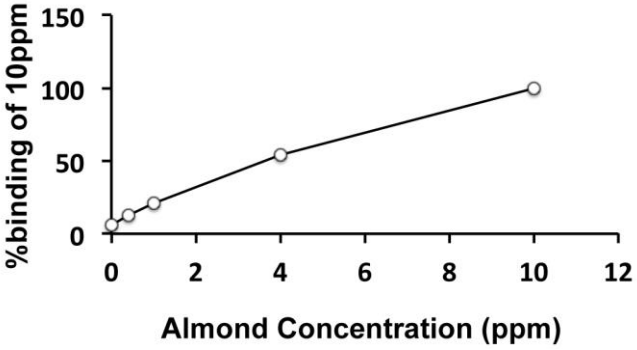
1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 100 µl of standards and samples in duplicate into wells.
3. Incubate for 20 minutes at RT.
4. Aspirate each well and wash, repeating the process 2 times for a total 3 washes. Wash by filling each well with 1X wash buffer (300 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
5. Add 100 µl of HRP-Antibody Conjugate into each well. Incubate for 20 minutes at RT.
6. Aspirate and wash well as step 4.
7. Add 100 µl of TMB mixture to each well. Incubate for 20 minutes at room temperature in dark.
8. Add 100 µl of Stop Solution to each well. The color of the solution should change from blue to yellow.
9. Read the OD with a microplate reader at 450 nm immediately. (optional reference absorbance at 620 nm).

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Using linear graph paper or semi-log graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay. The binding is calculated as percentage of the 10ppm standard.



QUALITY ASSURANCE

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

For the following foods with cross-reactivity could be detected:

Cherry kernel	1.7 %
Peach kernel	16 %
Plum kernel	1.0 %
Mahaleb cherry kernel	1.4 %

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 3-9% and inter-assay precision was 3-13%.

Recovery

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

Cookies	91%
Cereals	107%
Ice-cream	79%
Dark chocolate	69%

Intra-lot precision

2-8%

Linearity

85-98%