Cashew ELISA Kit

Enzyme Immunoassay for the quantitative determination of Cashew in food

Catalog number: ARG80791

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INTRODUCTION

The Cashew tree (Anacardium occidentale) belongs to the family of Anacardiaceae. With about 18 % the fraction of proteins in cashew seed is very high. Some of these proteins, like the vicilin Ana o 1, the legumin Ana o 2 or the albumin Ana o 3 are known for being allergenic. Many of them are heat resistant making them stable to different production processes. For this reason cashew represents an important food allergen. For cashew-allergic persons hidden cashew allergens in food are a critical problem. Already very low amounts of cashew can cause allergic reactions, which may lead to anaphylactic shock in severe cases. Because of this, cashew-allergic persons must strictly avoid the consumption of cashew containing food. Cross-contamination, mostly in consequence of the production process, is often noticed. This explains why in many cases the existence of cashew residues in food cannot be excluded. For this reason sensitive detection systems for cashew residues in foodstuffs are required. The Cashew ELISA represents a highly sensitive detection system for cashew and is particularly capable of the quantification of residues in cookies, cereals, ice-cream and chocolate.

PRINCIPLE OF THE ASSAY

This is an Enzyme Immunoassay for the quantitative determination of Cashew in food. This assay employs the sandwich quantitative enzyme immunoassay technique. An antibody specific for Cashew proteins is bound onto a pre-coated microtiter plate. Cashew 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 Cashew 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 Cashew. 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 1 ml (ready to use) (0,2,6,20,60 ppm)	4°C
10x Extraction & Sample dilution buffer	2X 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
- 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.
- Briefly spin down the HRP-Antibody conjugate before use.
- If crystals are observed in the 10X Wash buffer, Extraction Buffer and Sample dilution buffer, warm to RT (not more than 50°C) 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.

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 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 40°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.

The following sample preparation should be applied for liquid samples:

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

REAGENT PREPARATION

- 1X Wash buffer: Dilute 10X wash buffer into distilled water to yield 1X wash buffer.
- 1X Extraction and Sample Dilution Buffer: Dilute 5X Extraction buffer into distilled water to yield 1X Extraction buffer.

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 (350 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.
9. Read the OD with a microplate reader at 450 nm immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

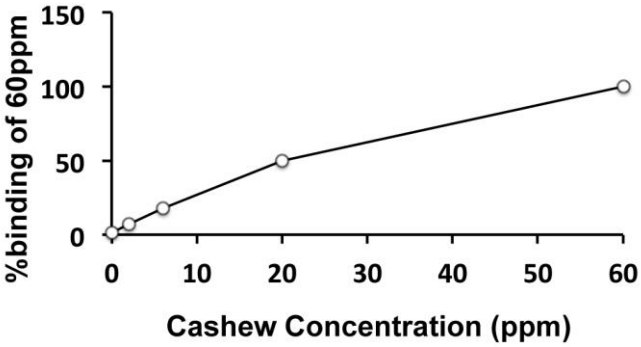
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

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 absorption of 60ppm standard.



QUALITY ASSURANCE

Sensitivity

The limit of detection (LOD) of the Cashew test is 0.2 ppm.

The limit of quantification (LOQ) of the Cashew 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:

Almond	Cow's milk	Pork meat
Apricot	Egg	Potato
Barley	Gliadin	Pumpkin seed
Bean	Goat's milk	Rice
Beef	Guar gum	Rye
Bovine gelatin	Hazelnut	Saccharose
Brazil nut	Kiwi	Sesame
Buckwheat	Lentil	Sheep's milk
Carob gum	Lupin	Shrimps, cooked
Chickpea	Macadamia	Shrimps, raw
Carrot	Mustard	Soy
Celery	Oats	Soy Lechithin
Chestnut	Pea	Sunflower seed
Chicken	Peanut	Tomato
Cocoa	Pecan	Walnut
Coconut	Pine seed	Wheat
Cod	Plum	
Corn	Poppy seed	

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 4-5% and inter-assay precision was 3-6%.

Recovery

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

Cookies	109%
Cereals	98%
Ice cream	93%
Dark chocolate	102%