Coconut ELISA Kit

Enzyme Immunoassay for the quantitative determination of Coconut in food

Catalog number: ARG81031

For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

The Cocos palm (Cocos nucifera) belongs to the family of Areaceae. With about 3.3% the fraction of proteins in coconut is relatively low compared to other nuts. Some of these proteins are known for being allergenic. Compared to other nuts these proteins are still slightly characterized. Coconut allergies are relatively seldom, but can be very distinct in particular cases.

For coconut-allergic persons hidden coconut allergens in food are a critical problem. Already very low amounts of coconut can cause allergic reactions, which may lead to anaphylactic shock in severe cases. Because of this, coconut-allergic persons must strictly avoid the consumption of coconut containing food. Cross-contamination, mostly in consequence of the production process, is often noticed.

This explains why in many cases the existence of coconut residues in food cannot be excluded. For this reason sensitive detection systems for coconut residues in foodstuff are required.

PRINCIPLE OF THE ASSAY

This assay employs the sandwich quantitative enzyme immunoassay technique. An antibody directed against coconut proteins is bound on the surface of a microtiter plate. Coconut 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 coconut 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 color. The color development is inhibited by the addition of a stop solution, and the color turns yellow. The yellow color is measured at 450 nm. The concentration of coconut is directly proportional to the color intensity of the test sample.

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 (0,2, 5, 15, 30 ppm)	5 X 2 ml (ready to use)	4°C
10x Extraction and 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 450 nm and 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.
- Briefly spin down the HRP-Antibody conjugate before use.
- If crystals are observed in the 10X Wash buffer, Extraction Buffer and Sample diluent buffer, warm to RT or 37°C for 15 min or until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Once the assay has been started, all subsequent steps should be completed without interruption and within the recommended time limits.
- Replace caps in all the reagents immediately after use. Do not interchange vial stoppers.
- The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance
- 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. Coconut proteins might 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 all 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 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. (E.g. 60 ml of 10X Wash buffer + 540 ml of distilled water) The diluted Wash buffer is stable for at least one week at 2°C to 8°C.
- **1X Extraction and Sample diluent buffer:** Dilute 10X Extraction and Sample diluent buffer into distilled water to yield 1X Extraction and Sample diluent buffer. (E.g. 50 ml of 10X Extraction and Sample diluent buffer + 450 ml of distilled water) The diluted Extraction and Sample diluent buffer is stable for at least one week at 2°C to 8°C.
- **Sample:** If the initial assay found samples contain Coconut higher than the highest standard, the samples can be diluted with 1X Extraction and Sample diluent buffer and then re-assay the samples. For the calculation of the concentrations this dilution factor has to be taken into account.
- **Standards:** In any case the ready-to-use standards provided should be determined in duplicates. 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.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-25°C) before use. Standards, samples and controls should be assayed in duplicates.

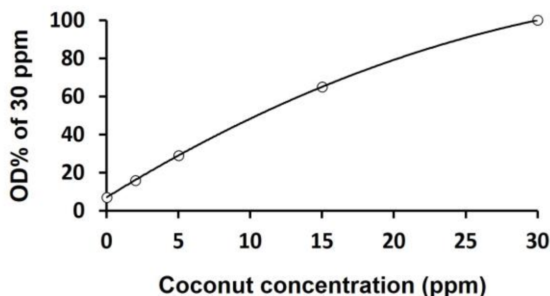
1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 100 µl of standards and prepared samples in duplicate into wells of the antibody coated-microtiter plate..
3. Incubate for 20 minutes at RT.
4. Aspirate each well 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, gently tap the plate to mix well. The color of the solution should change from blue to yellow.
9. Read the OD with a microplate reader at 450 nm (reference wavelength 620 nm) immediately. It is recommended read the absorbance within 30 min after adding STOP solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Using log-log, semi-log or 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. The diluted samples must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.
5. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

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.



QUALITY ASSURANCE

Sensitivity

The limit of detection (LOD) of the Coconut test is 0.4 ppm.

The limit of quantification (LOQ) of the Coconut test is 2 ppm.

Validation experiments with common matrices resulted in the following LODs

Cookies: 0.4 ppm

Cornflakes: 0.2 ppm

Ice-cream: 0.3 ppm

Chocolate: 0.5 ppm

Sausage: 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:

Almond	Egg	Pine seed
Apricot	Egg white powder	Pistachio
Barley	Ewe's milk	Plum
Bean, white	Gelatin	Poppy seed
Beef	Gliadin	Pork
Brazil nut	Goat's milk	Potato
Buckwheat	Hazelnut	Pumpkin seed
Carob gum	Peanut	Rice
Carrot	Isinglass	Rye
Cashew	Kiwi	Saccharose
Celery	Lentil	Sesame
Cherry	Lupin	Shrimp, cooked
Chervil	Macadamia nut	Shrimp, raw
Chestnut	Milk	Soy
Chick pea	Mustard	Soy lecithin
Chicken	Oats	Sunflower seed
Cocoa	Pea	Tofu
Cod	Peach	Tomato
Corn	Peanut	Walnut
Cress	Pecan	Wheat

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 4% and the CV value of inter-assay precision was 6-10%

Linearity

The serial dilution of spiked samples (cookies, cereals, ice-cream, chocolate and sausage) resulted in a dilution linearity of 102-125%.

Recovery

Cookies	92%
Cornflakes	102%
Ice-cream	74%
Chocolate	87%
Sausage	80%