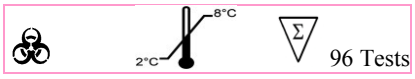
AccuDiag™ Coconut ELISA

REF 5160-8



Test	Coconut ELISA
Recovery	74 - 102%
Incubation Time	60 min
Sensitivity (Coconut)	0.4 ppm

INTENDED USE

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.

The DAI Coconut ELISA represents a highly sensitive detection system for coconut and is particularly capable of the quantification of residues in cookies, cereals, ice-cream, chocolate and sausage.

TEST PRINCIPLE

The DAI Coconut quantitative test is based on the principle of the enzyme linked immunosorbent assay. 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 terminated by the addition of a stop solution, and the color turns yellow. The yellow color is measured photometrically at 450 nm. The concentration of coconut is directly proportional to the color intensity of the test sample.

MATERIALS AND COMPONENTS

Materials provided with the test kits

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-coconut antibodies.
2. Coconut Standards (0, 2, 5, 15, 30 ppm of coconut): 5 vials with 4.0 mL each, dyed red, ready-to-use.
3. Conjugate (anti-coconut-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-to-use.
6. Extraction and sample dilution buffer (Tris): 2 x 120 mL as 10x 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 provided

Instrumentation

1. 100 - 1000 µL micropipettes
2. Volumetric flask
3. Analytical balance
4. Mortar, mixer
5. Water bath
6. Centrifuge
7. ELISA reader (450 nm)

Reagents

Double-distilled water

SAMPLE PREPARATION

Due to the 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 representative-ness 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.

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.

PRECAUTION

Full compliance of the following good laboratory practices (GLP) will determine the reliability of the results:

1. Prior to beginning the assay procedure, bring all reagents to room temperature (20-25°C).
2. All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
3. Once the assay has been started, all subsequent steps should be completed without interruption and within the recommended time limits.
4. Replace caps in all the reagents immediately after use. Do not interchange vial stoppers.
5. Use a separate disposable tip for each specimen to prevent cross-contamination.
6. All specimens and standards should be run at the same time, so that all conditions of testing are the same.
7. Do not mix components from different batches.
8. Do not use reagents after expiration date.
9. Check both precision and accuracy of the laboratory equipment used during the procedure (micropipettes, ELISA reader etc.).

HEALTH & SAFETY INSTRUCTIONS

1. Do not smoke or eat or drink or pipet by mouth in the laboratory.
2. Wear disposable gloves whenever handling patient specimens.
3. Avoid contact of substrate and stop solution with skin and mucosa (possible irritation, burn or toxicity hazard). In case of contact, rinse the affected zone with plenty of water.
4. Handling and disposal of chemical products must be done according to good laboratory practices (GLP).

ASSAY PROCEDURE

The washing solution is supplied as 10x concentrate and has to be **diluted** 1+9 with double distilled water before use.

In any case the **ready-to-use** standards 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 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-coconut-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 color will turn yellow upon addition.
11. After thorough mixing, measure absorbance at 450 nm (reference wavelength 620 nm), using an ELISA reader. The color is stable for 30 minutes.

RESULTS

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 coconut in ppm from the standard curve. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed.

Typical Standard Values

The following table contains an example for a typical standard curve. The OD% 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.

Coconut (ppm)	OD% of 30 ppm
30	100
15	65
5	29
2	16
0	7

PERFORMANCE CHARACTERISTICS

Sensitivity

The limit of detection (LOD) of the **DAI Coconut ELISA** test is 0.4 ppm for the standard curve.

Validation experiments with common matrices resulted in the following LODs (ppm).

Cookies	0.4
Cornflakes	0.2
Ice cream	0.3
Chocolate	0.5
Sausage	0.4

The limit of quantification (LOQ) of the **DAI Coconut ELISA** 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.

Cross-reactivity
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	Guar flour	Pumpkin seed
Carob gum	Hazelnut	Rice
Carrot	Isinglass	Rye
Cashew	Kiwi	Sucrose
Celery	Lentil	Sesame
Cherry	Lupine	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

Precision

Intra-assay Precision	4%
Inter-assay Precision	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
Mean recovery was determined by spiking samples with different amounts of coconut:

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

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