



# Macadamia nut ELISA Kit

Enzyme Immunoassay for the quantitative determination of Macadamia nut  
in food

Catalog number: ARG81032

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

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### INTRODUCTION

The Macadamia tree (*Macadamia integrifolia*, *Macadamia tetraphylla*) belongs to the family of Proteaceae. With about 7.9% the fraction of proteins in macadamia nut 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. Macadamia nut allergies are relatively seldom, but can be very distinct in particular cases.

For macadamia nut allergic persons hidden macadamia nut allergens in food are a critical problem. Already very low amounts of macadamia nut can cause allergic reactions, which may lead to anaphylactic shock in severe cases. Because of this, macadamia nut-allergic persons should strictly avoid the consumption of macadamia nut containing food. Cross-contamination, mostly in consequence of the production process, is often noticed. This explains why in many cases the existence of macadamia nut residues in food cannot be excluded. For this reason sensitive detection systems for macadamia nut residues in foodstuff are required.

This Macadamia nut ELISA represents a highly sensitive detection system for macadamia nut and is particularly capable of the quantification of residues in cookies, cereals, ice-cream, chocolate and sausage.

### PRINCIPLE OF THE ASSAY

This assay employs the sandwich quantitative enzyme immunoassay technique. An antibody directed against Macadamia nut proteins is bound on the surface of a microtiter plate. Macadamia nut containing samples or standards are given

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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 Macadamia nut 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 Macadamia nut 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, 1, 4, 10, 40 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 (read at 620 nm as reference wavelength).
- 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 or 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

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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. Macadamia nut 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.

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4. 100  $\mu$ L 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 4 weeks 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:** *The dilution of samples in the extraction process as described in the above stated sample preparation procedure is already considered.* If the initial assay found samples contain Macadamia nut higher than the highest standard, the samples can be diluted with 1X Extraction and

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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 the appropriate 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. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbances.
5. Add 100 µl of HRP-Antibody Conjugate into each well. Incubate for 20 minutes at RT.



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6. Aspirate and wash well as step 4.
7. Add 100  $\mu$ l of TMB substrate to each well. Incubate for 20 minutes at room temperature in dark.
8. Add 100  $\mu$ l of Stop Solution to each well, gently tap the plate to mix well. The color of the solution should change from blue to yellow. Gently tap the plate to ensure thorough mixing.
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.

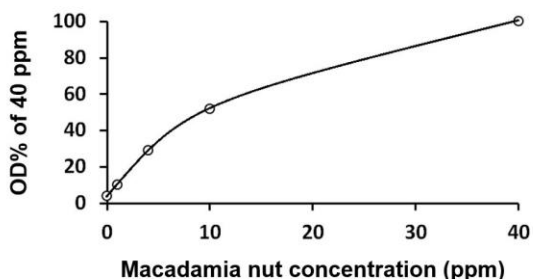
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5. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

### 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 Macadamia nut test is 0.1 ppm.

The limit of quantification (LOQ) of the Macadamia nut test is 1 ppm.

Validation experiments with common matrices resulted in the following LODs

Cookies: 0.2 ppm

Cornflakes: 0.1 ppm

Ice-cream: 0.2 ppm

Chocolate: 0.4 ppm

Sausage: 0.1 ppm

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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	Corn	Peach
Apricot	Cow' milk	Peanut
Barley	Crab, cooked	Pecan
Bean, white	Crab, rob	Pine seed
Beef	Cress	Pistachio
Bovine gelatin	Egg	Plum
Brazil nut	Egg white powder	Poppy seed
Buckwheat	Ewe's milk	Pork
Carob gum	Fish gelatin	Potato
Carrot	Gliadin	Pumpkin seed
Cashew	Goat's milk	Rice
Celery	Guar gum	Rye
Cherry	Isinglass	Saccharose
Chervil	Kiwi	Sesame
Chestnut	Lamb	Soy
Chick pea	Lentil	Soy lecithin
Chicken	Lupin	Sunflower seed
Cocoa	Mustard	Tofu
Coconut	Oats	Tomato
Cod	Pea	Wheat

The following cross reactions were determined:

Hazelnut	0.0002%
Walnut	0.0010%

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### Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 2-5% and the CV value of inter-assay precision was 3-6%

### Linearity

The serial dilution of spiked samples (cookies, cereals, ice-cream, chocolate and sausage) resulted in a dilution linearity of 101-108%.

### Recovery

Cookies	106%
Cornflakes	104%
Ice-cream	92%
Chocolate	83%
Sausage	101%