Walnut ELISA Kit

Enzyme Immunoassay for the quantitative determination of Walnut in food

Catalog number: ARG80818

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

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INTRODUCTION

Walnut (Juglans regia) belongs to the walnut plants (Juglandaceae). With 15% the fraction of proteins in walnuts is high. Some of these proteins are known for being allergenic, such as rJug r 1 and rJug r 4. Many of them are heat resistant making them stable to different production processes. For this reason walnut represents an important food allergen. For walnut allergic persons hidden walnut allergens in food are a critical problem. Already very low amounts of walnut can cause allergic reactions, which may lead to anaphylactic shock in severe cases. Because of this, walnut allergic persons must strictly avoid the consumption of walnuts or walnut containing food. Cross-contamination, most-ly 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 walnut residues in food cannot be excluded. For this reason sensitive detection systems for walnut residues in food-stuffs are required.

The Walnut ELISA Kit represents a highly sensitive detection system and is particularly capable of the quantification of walnut residues in cookies, cereals, ice cream and chocolate.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative enzyme immunoassay technique. An antibody directed against walnut proteins is bound on the surface of a microtiter plate. Walnut 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 walnut 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 walnut 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
Standard 1-5 (0, 2, 6, 20, 60 ppm)	5 X 2 ml (Ready to use)	4°C
10X Extraction and sample dilution buffer	2 x 120 ml	4°C
HRP-antibody conjugate	15 ml (Ready to use)	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 450/620nm
- Pipettes and pipette tips
- Mortar, mixer
- Volumetric flask
- Analytical balance
- Water bath
- Deionized or double 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.
- All specimens and standards should be run at the same time, so that all conditions of testing are the same.
- Do not mix components from different batches.
- Do not use reagents after expiration date.
- If crystals are observed in the 10X Wash buffer and Extraction and sample dilution buffer, warm to 37°C 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.
- Prior to beginning the assay procedure, bring all reagents to room temperature (20-25°C).
- 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. Walnut proteins adhere very strongly 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 all kinds 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.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 10X wash buffer into double distilled water to yield 1X wash buffer. (e.g. add 30 ml of 10X wash buffer into 270 ml of double distilled water, mix well) Store the diluted buffer at 4°C the diluted buffer is stable for at least one week. If crystals are observed in the Wash buffer warm to 37°C for 15 min or until the crystals are completely dissolved.
- **1X Extraction and Sample dilution buffer:** Dilute 10X Extraction and Sample dilution buffer into double distilled water to yield 1X Extraction and Sample dilution buffer. (e.g. add 30 ml of 10X Extraction and Sample dilution buffer into 270 ml of double distilled water, mix well) Store the diluted buffer at 4°C the diluted buffer is stable for at least 4 weeks. If crystals are observed in the Extraction and Sample dilution buffer, warm to 37°C for 15 min or until the crystals are completely dissolved.

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.

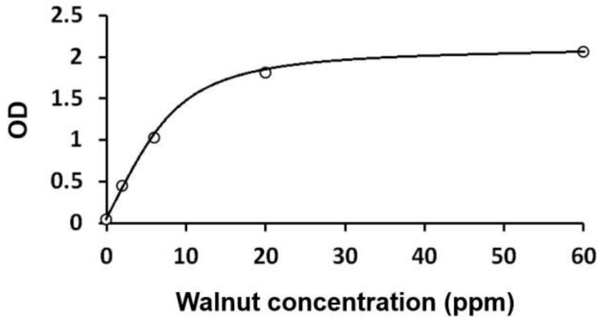
1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **100 µl** of **standards and samples** in duplicate into wells.
3. Incubate for **20 minutes** at RT.
4. Aspirate each well and wash, repeating the process 2 times 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 substrate** 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. 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 minutes** after adding the 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 further 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 Walnut test is 0.35 ppm.

The limit of quantification (LOQ) of the Walnut 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.

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 4-9% and the CV value of inter-assay precision was 12%.

Linearity

The serial dilution of spiked samples (cookies, cereals, ice cream and chocolate) resulted in a dilution linearity of 70% - 128%.

Recovery

Cookies	103%
Cereals	106%
Ice cream	86%
Chocolate	75%

Specificity

The Cross-reactivity of other egg white proteins as well as potential fining reagents was determined as follows:

Adzuki	Almond	Apricot	Barley
Bean, white	Beef	Bovine gelatin	Brazil nut
Buckwheat	Caraway	Carob gum	Carrot
Cayenne	Celery	Cherry	Chervil
Chestnut	Chia	Chickpea	Chili
Cocoa	Coconut	Cod	Corn
Cow's milk	Crab, cooked	Crab, raw	Cress
Cumin	Duck	Egg	Ewe's milk
Fenugreek	Fish gelatin	Gliadin	Goat's milk
Guar gum	Hazelnut	Isinglass	Kidney bean
Kiwi	Lamb	Lentil	Lupin
Macadamia	Mustard	Nutmeg	Oats
Onion	Paprika	Pea	Peanut
Pepper	Pine seed	Pistachio	Plum
Poppy seed	Pork	Potato	Pumpkin seed
Rice	Rye	Saccharose	Sesame
Shrimp, cooked	Shrimp, raw	Soy flour	Sunflower seeds
Tomato	Turkey	Wheat	

The following cross reactions were determined:

Cashew	0.003 %
Chicken	0.003 %
Pecan	0.005 %