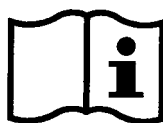


Product information

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User's Manual

Walnut ELISA

Enzyme Immunoassay for the quantitative determination of Walnut in food



DEWALE01



96 wells



For Research Use Only – Not for Use in Diagnostic Procedures

Sensitivity (Walnut)	0.35 ppm
Recovery	75-106%
Incubation Time	60 min

1. GENERAL INFORMATION

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

This test is designed for detection of substances within food products. This is not a medical device and is not intended to diagnose or prevent any diseases or other conditions.

2. PRINCIPLE OF THE TEST

The **Walnut** quantitative test is based on the principle of the enzyme linked immunosorbent assay. 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 colour. The colour development is inhibited by the addition of a stop solution, and the colour turns yellow. The yellow colour is measured photometrically at 450 nm. The concentration of walnut is directly proportional to the colour intensity of the test sample.

3. PRECAUTIONS

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 (micropipets, ELISA reader etc.).

4. HEALTH AND 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).

5. REAGENTS

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. **SORB MT** Microtiter plate consisting of 12 strips with 8 breakable wells each, coated with anti-walnut antibodies.
2. **CAL 1 – 5** Walnut Standards (0; 2; 6; 20; 60 ppm of walnut): 5 vials with 2.0 mL each, dyed red, ready-to-use.

3. **ENZ** **CONJ** Conjugate (anti-walnut-peroxidase): 15 mL, dyed red, ready-to-use.
4. **SUB** **TMB** Substrate Solution (TMB): 15 mL, ready-to-use.
5. **STOP** **SOLN** Stop Solution (0.5 M H₂SO₄): 15 mL, ready-to-use.
6. **SAM** **DIL** **10x** 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. **WASH** **SOLN** **10x** 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. Instruction Manual.

6. ADDITIONAL INSTRUMENTATION AND REAGENTS (NOT PROVIDED)

Instrumentation

- 100 - 1000 µL micropipets
- Volumetric flask
- Analytical balance
- Mortar, mixer
- Water bath
- Centrifuge
- ELISA reader (450 nm)
- Plastic bag to store unused microtiter strips.

Reagents

- double distilled water

7. SAMPLE PREPARATION

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 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.

8. 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 provided 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-walnut-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 H₂SO₄) into each well. The blue colour will turn yellow upon addition.
11. After thorough mixing, measure absorbance at 450 nm (reference wavelength 620 nm), using an ELISA reader. The colour is stable for 30 minutes.

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

10. TYPICAL STANDARD VALUES

The following table contains an example for a typical standard curve. The binding is calculated as percent of the absorption of the 60 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.

Walnut (ppm)	% binding of 60 ppm
60	100
20	80
6	55
2	32
0	12

11. PERFORMANCE

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.

Cross-reactivity

For the following foods no cross-reactivity could be detected:

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

The following cross reactions were determined:

Cashew	0.0003%
Chicken	0.0003%
Pecan	0.0005%

Precision

Intra-assay Precision	4 – 9%
Inter-assay Precision	12%

Linearity

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

Recovery












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

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

12. REFERENCES

1. Doi H, et al. (2008) – Reliable enzyme-linked immunosorbent assay for the determination of walnut proteins in processed foods. J Agric Food Chem, 56(17):7625-30
2. Wallowitz M, et al. (2006) – Jug r 4, a legumin group food allergen from walnut (Juglans regia Cv. Chandler). J Agric Food Chem, 54(21):8369-15
3. Niemann L, et al. (2009) – Detection of walnut residues in foods using an enzyme-linked immunosorbent assay. J Food Sci, Published Online 10 Jun 2009
4. Goetz DW, et al. (2005) – Cross-reactivity among edible nuts: double immunodiffusion, crossed immunoelectrophoresis and human specific IgE serologic surveys. Ann All Asthma Immunol, 95(1):45-52
5. Roux KH, et al. (2003) – Tree nut allergens. Int Arch Allergy Immunol, 131(4):234-44

SYMBOLS USED WITH DEMEDITEC ASSAYS

Symbol	English	Deutsch	Francais	Espanol	Italiano
	European Conformity	CE-Konformitätskennzeichnung	Conforme aux normes européennes	Conformidad europea	Conformità europea
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las Instrucciones	Consultare le istruzioni per l'uso
	In vitro diagnostic device	In-vitro-Diagnostikum	Ussage Diagnostic in vitro	Diagnóstico in vitro	Per uso Diagnostica in vitro
	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
	Catalogue number	Katalog-Nr.	Référence	Número de catálogo	No. di Cat.
	Lot. No. / Batch code	Chargen-Nr.	No. de lot	Número de lote	Lotto no
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Note warnings and precautions	Warnhinweise und Vorsichtsmaßnahmen beachten	Avertissements et mesures de précaution font attention	Tiene en cuenta advertencias y precauciones	Annoti avvisi e le precauzioni
	Storage Temperature	Lagerungstemperatur	Temperature de conservation	Temperatura de conservacion	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeitsdatum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
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