

# Product information

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## User's Manual

# Brazil Nut ELISA

Enzyme immunoassay for the quantitative determination of Brazil nut in food

**REF**

**DEPARE01**



**96 wells**

**RUO**

**For Research Use Only – Not for Use in Diagnostic Procedures**

Sensitivity (Brazil nut)	0.2 ppm
Recovery	78-114%
Incubation Time	60 min

## 1. GENERAL INFORMATION

The **Demeditec Brazil nut ELISA** represents a highly sensitive detection system for brazil nut and is particularly capable of the quantification of residues in cookies, cereals, ice-cream, chocolate and sausage.

*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 **Demeditec Brazil nut** quantitative test is based on the principle of the enzyme linked immunosorbent assay. An antibody directed against brazil nut proteins is bound on the surface of a microtiter plate. Brazil nut 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 brazil 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 colour. The colour development is terminated by the addition of a stop solution, and the colour turns yellow. The yellow colour is measured photometrically at 450 nm. The concentration of brazil nut 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 printed 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-brazil nut antibodies.
2. **CAL 1 – 5** Brazil nut Standards (0, 1, 4, 10, 40 ppm of brazil nut): 5 vials with 2.0 mL each, dyed red, ready-to-use.
3. **ENZ CONJ** Conjugate (anti-brazil nut-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 H<sub>2</sub>SO<sub>4</sub>): 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. Brazil nut proteins could adhere 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 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 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.

## 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 absorbances.
5. Pipet 100 µL of conjugate (anti-brazil nut-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<sub>2</sub>SO<sub>4</sub>) 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 (OD) value for each sample, determine the corresponding concentration of brazil nut in ppm from the standard curve. Depending on experience and/or the availability of computer software, other methods of data reduction may be employed.

## 10. 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 40 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.

Brazil nut (ppm)	OD% of 40 ppm
40	100
10	58
4	28
1	10
0	3

## 11. PERFORMANCE

### Sensitivity

The limit of detection (LOD) of the **Demeditec Brazil nut test** is 0.2 ppm for the standard curve. Validation experiments with common matrices resulted in the following LODs [ppm].

Cookies	0.3
Cornflakes	0.1
Ice-cream	0.2
Chocolate	0.2
Sausage	0.2

The limit of quantification (LOQ) of the **Demeditec Brazil nut test** is 1 ppm. Due to the variety of sample matrices and their influence on the blank, results less than the LOQ should be treated as negative.

### Precision

Intra-assay Precision	8%
Inter-assay Precision	6-11%

### Linearity

The serial dilution of spiked samples (cookies, cereals, ice-cream, chocolate and sausage) resulted in a dilution linearity of 86-96%.

### Cross-reactivity

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

Almond	Chevil	Ewe's milk	Oats	Rapeseed
Apricot	Chicken	Fish gelatin	Onion	Rice
Barley	Chickpea	Gliadin	Paprika	Rye
Bean, white	Cocoa	Goat's milk	Pea	Saccharose
Beef	Coconut	Guar gum	Peach	Sesame
Bovine gelatin	Cod	Hazelnut	Peanut	Shrimp, cooked
Buckwheat	Corn	Isinglass	Pepper	Shrimp, raw
Caraway	Cow' milk	Kiwi	Pine seed	Soy
Carob gum	Crab, cooked	Lamb	Pistachio	Soy lecithin
Carrot	Crab, raw	Lentil	Plum	Sunfl. seeds
Cashew	Cress	Lupin	Poppy seed	Tofu
Cayenne	Cumin	Macadamia	Pork	Tomato
Celery	Egg	Mustard	Potato	Walnut
Cherry	Egg white powder	Nutmeg	Pumpkin seed	Wheat
Chestnut				

The following cross reactions were determined:

Pecan nut	0.0002%
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**Recovery**

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

Cookies	93%
Cornflakes	112%
Ice-cream	107%
Chocolate	76%
Sausage	90%

**12. REFERENCES**

1. Feng G, et al. (2007) – Purification, crystallization and initial crystallographic characterization of brazil-nut allergen Ber e 2. Acta Cryst, 63:976-979
2. Girdhari M, et al. (2009) – A sensitive and robust competitive enzyme-linked immunosorbent assay for brazil nut (*Bertholletia excelsa*) detection. J Agric Food Chem, 57:769-776

## SYMBOLS USED WITH DEMEDITEC ASSAYS

Symbol	English	Deutsch	Français	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	Usage 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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