Version 09-03/25

User's Manual

Brazil Nut ELISA Kit

REF **DEIA-FA001**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

Enzyme Immunoassay for the Quantitative Determination of Brazil Nut in food.

General Description

Brazil nuts are 14% protein, 12% carbohydrate, and 66% fat by weight; 85% of their calories come from fat, and a 100 gram amount provides 656 total calories. The fat components are 23% saturated, 38% monounsaturated, and 32% polyunsaturated. Due to their high polyunsaturated fat content, primarily omega-6 fatty acids, shelled Brazil nuts may quickly become rancid.

Principles of Testing

Sandwich enzyme immunoassay

Reagents And Materials Provided

1. Microtiter plate consisting of 12 strips with 8 breakable wells each, coated with anti-almond antibodies.
2. Standards : 5 vials, ready-to-use
3. Conjugate: 15 mL, ready-to-use.
4. Substrate Solution (TMB): 15 mL, ready-to-use.
5. Stop Solution (0.5 M H2SO4): 15 mL, ready-touse.
6. Extraction and sample dilution buffer (Tris)
7. Washing Solution (PBS + Tween 20): 60 mL as 10x concentrate.
8. Plastic bag to store unused microtiter strips.
9. Instruction Manual.

Materials Required But Not Supplied

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

Storage

Stored at 2-8°C.

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 provided should be determined two fold. 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 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 solu tion (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.

Detection Limit

0.2 ppm

Specificity

Pecan nut 0.0002%