Brazil Nut ELISA Kit

Enzyme Immunoassay for the quantification determination of Brazil Nut in food

Catalog number: ARG83050

Package: 96 wells

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MANUFACTURED BY:

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INTRODUCTION

Allergies to tree nuts such as Brazil nuts are common and often severe. These types of allergies typically develop by the age of 2, and the number of tree nuts to which a person is allergic may increase with age. Roughly 30 percent of people with a tree nut allergy are allergic to more than one nut. And while peanuts are actually legumes, approximately 20 to 30 percent of those with a peanut allergy are also allergic to one or more types of tree nuts. In fact, together, peanuts and tree nuts account for 70 to 90 percent of reported food-related anaphylactic fatalities. Prevalence for tree nut allergy varies by age, region, and the definitions used for diagnosis, but it appears to affect 0.05 to 7.3 percent of the population. And unfortunately, compared to other food allergies, the chances of outgrowing these allergies are lower and restricted to an estimated 10 percent of sensitized individuals. The following nine varieties account for the majority of tree nut allergies: walnuts, almonds, pistachios, cashews, pecans, hazelnuts, macadamias, Brazil nuts, and pine nuts. Also called a Para nut, Brazil nuts are actually an edible seed from a South American tree that produces hard-walled fruits resembling coconuts, which are roughly 8 to 18 centimeters (roughly 3 to 7 inches) in diameter. Weighing up to approximately 2.25 kilograms (5 pounds), these fruits typically contain 12 to 24 seeds (aka Brazil nuts) arranged like sections of citrus fruits. The fruits ripen and fall to the ground, where they're harvested. Then, the three-sided seeds are removed, dried, washed, and sold while still in their shells. Brazil nuts are high in protein, dietary fiber, thiamin, copper, and magnesium. However, compared to other tree nuts, they're the best source of selenium, which among other things is involved in modulating the immune system, regulating

the thyroid, and protecting against oxidative stress. Brazil nut oil has been used in shampoos, soaps, hair conditioners, and skin-care products, and the nuts are eaten raw or blanched and are most often found in mixed nut offerings. In the United Kingdom, sensitization to Brazil nuts affects 24 to 33 percent of those with tree nut allergies, but in the United States, the reported figure is less than 5 percent.

PRINCIPLE OF THE ASSAY

This assay employs the sandwich quantitative enzyme immunoassay technique. 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 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 Brazil Nut proteins 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	8 X 12 strips	4°C. Unused strips should be sealed tightly in the air-tight pouch.
Standard A-E (0, 1, 4, 10, 40 ppm)	5 X 2 ml (Ready-to-use)	4°C.
10X Sample Diluent concentrate	2 X 120 ml	4°C.
HRP-Streptavidin Buffer	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 450nm
- 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 in the dark at all times. Do not expose reagents to heat, sun, or strong light during storage and usage.

- Diluted Wash Buffer and Sample Buffer are stable for at least 30 days when stored at 2-8°C. We recommend consumption on the same day.
- Briefly spin down the antibody conjugate Mixture and HRP-Streptavidin concentrate before use.
- All materials must be at room temperature (20-28°C) prior to use.
- If crystals are observed in the 20X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- All incubation steps must be accurately timed.
- Change pipette tips between the addition of different reagent or samples.

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. Brazil Nut 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 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 prediluted 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 2500 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 prediluted 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 distilled water to yield 1X Wash buffer. (E.g. 10 ml of 10X Wash buffer + 90 ml of distilled water) The diluted Wash buffer is stable for 30 days at 2°C to 8°C.

- 1X Sample Diluent buffer: Dilute 10X Sample Diluent concentrate into distilled water to yield 1X Sample Diluent buffer. (E.g. 10 ml of 10X Sample Diluent concentrate + 90 ml of distilled water) The Sample diluent buffer is stable for 7 days at 2°C to 8°C.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-28 °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** 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 **1× Wash Buffer (300µl)** 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-Streptavidin Buffer** into wells.
6. Incubate for 20-25 minutes at RT.
7. Aspirate and wash well as step 4.
8. Add **100 µl** of **TMB Reagent** to each well.
9. Incubate for 20-25 minutes at RT in dark.
10. Add **100 µl** of **Stop Solution** to each well. The color of the solution should change from blue to yellow.
11. Read the OD with a microplate reader at **450 nm** immediately (optional: Read the OD at 600-690nm as reference). 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 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.

5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)

6. If these quality control criteria are not met the assay run is invalid and should be repeated.

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 standard range of Ochratoxin A ranged from 1-40 ppm. The mean Limit of detection was 0.2 ppm.

Cross-reactivity

Pecan nut < 0.001%

Hazelnut < 0.001%

Sesame < 0.001%

Linearity

94%- 101%.

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 8.2% and inter-assay precision was 3.7%.