Peanut ELISA Kit

Enzyme Immunoassay for the quantitative determination of Peanut in food

Catalog number: ARG80809

Package: 96 wells

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INTRODUCTION

Peanut (Arachis hypogaea) belongs to the legumes. With 25 % the fraction of proteins in peanuts is very high. Many of these proteins are known for being aller-genic, such as Arachins and Conarachins which are contained in relative high amounts. For this reason peanut represents one of the most important food allergens. For peanut allergic persons hidden peanut allergens in food are a critical problem. Already very low amounts of peanuts can cause allergic reactions, which may lead to anaphylactic shock in severe cases. Because of this, peanut allergic per-sons must strictly avoid the consumption of peanuts or peanut containing food. Cross-contamination, most-ly in consequence of the production pro-cess is often noticed. The chocolate production pro-cess is a repre-sentative example. This explains why in many cases the existence of peanut residues in foods can-not be excluded. For this reason sensitive detection systems for peanut residues in food-stuffs are required.

PRINCIPLE OF THE ASSAY

This assay employs the sandwich quantitative enzyme immunoassay technique. An antibody directed against peanut proteins is bound on the surface of a microtiter plate. Peanut containing samples or standards are given in-to 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 peanut 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 peanut 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	12 strips x 8-well	4°C
HRP-antibody Conjugate	15 ml (ready to use)	4°C
Standards A-E (0, 1, 4, 10, 40 ppm)	5 X 2 ml	4°C
10x Extraction and sample dilution buffer	2 X 120 ml	4°C
10x Wash Buffer	60 ml	4°C
TMB substrate	15 ml	4°C (Protect from light)
STOP solution	15 ml	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 at all times.
- Briefly spin down the HRP-Antibody conjugate before use.
- If crystals are observed in the 10X Wash buffer, Extraction Buffer and Sample diluent buffer, warm to RT (not more than 50°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.
- 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. Hazelnut proteins 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 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 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 pre¬heated water bath at 60°C. To ensure good homo¬geneity, the samples should be shaken every¬ two minutes. The process is continued at point 3 of solid sample extraction process.

Note:

a) Do not shake the final extract to prevent from re-suspension.
b) If after centrifugation a third layer at the top appears due to a high fatty matrix, only the middle aqueous phase should be applied to the wells.

REAGENT PREPARATION

- **1X Wash buffer**: Dilute 10X wash buffer into distilled water to yield 1X wash buffer.
- **1X Extraction and Sample diluent buffer:** Dilute 10X Extraction and Sample diluent buffer into distilled water to yield 1X.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) 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.
9. Read the OD with a microplate reader at 450 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 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.

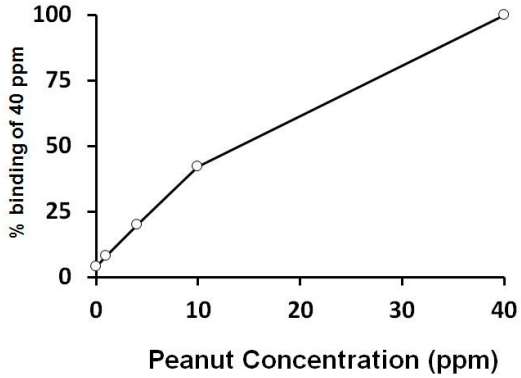
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

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. 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)

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 Peanut test is 0.1 ppm.

The limit of quantification (LOQ) of the Peanut 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.

Specificity

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

Adzuki bean	Goat's milk	Chicken
Pine seed	Almond	Tomato
Chickpea	Pistachio	Guar gum
Turkey	Chili	Apple

Hazelnut	Turmeric	Poppy
Apricot	Horseradish	Cinnamon
Pork	Barley	Walnut
Coconut	Potato	Kidney bean
Wheat	Cod	Bean, white
Kiwi	Beef, cooked	Prawn cooked
Corn	Prawn, raw	Lamb
Beef, raw	Leek	Cow's milk
Pumpkin seed	Cumin	Bovine gelatin
Lentil	Brazil nut	Radish
Dill	Rapeseed	Lupin
Buckwheat	Macadamia	Duck
Rice	Egg	Cabbage, white
Milk powder	Caraway	Rye
Fennel	Saccharose	Mustard
Cardamom	Nutmeg	Fenugreek
Sesame	Flaxseed	Carob gum
Oats	Carrot	Shrimps
Garden cress	Soy flour	Onion
Cashew	Paprika	Garlic, fresh
Soy lecithin	Garlic, granulated	Celery
Pea	Cherry	Split pea
Ginge, ground	Sunflower seed	Peach
Chestnut	Pecan	Ginger, fresh
Thyme	Gliadin	Chia

Pepper, black	Tofu	

For the following commodities of the table above the results were between 0.5*LOQ and LOQ of the kit. So, it cannot be completely excluded that these matrices may provide values above the LOQ in specific cases:

Soy flour	Almond	Fenugreek
Carob gum	Pecan	Lentil

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 7-10% and the CV value of inter-assay precision was 2-11%

Recovery

Cookies	97%
Hazelnut	96%
Cereals	102%
Ice cream	93%
Chocolate	96%