

## PEANUT DIAGNOKIT™

The ingestion of certain proteins may result in serious allergic reactions in hypersensitive individuals whether children or adults. Reactions vary from simple urticaria to fatal anaphylaxis and only avoidance may be an effective means of protecting consumers.

Reactions to peanut is one of the most prevalent food allergy affecting a substantial proportion of the population. About 20% of the hypersensitive individuals are reported to be allergic to peanuts. With the current use of peanuts and peanut by-products as supplements in many types of foods, cross-contamination may occur: in a five year period over 50% of the patients experienced at least one accidental ingestion of peanut proteins due to poor labeling of food products.

Ara hI (63.5 kDa), Ara hII (17 kDa) and agglutinin (31 kDa) have been identified as the major peanut allergens incriminated in IgE related reactions.

### **Peanut DiagnoKit™:**

Quality control methodologies are required to identify whether peanut allergens have made their way to the manufactured food products with no proper labeling. They are also useful to consumer protection agencies in order to enforce existing regulations on labeling of food ingredients.

Peanut DiagnoKit™ is a competitive immunoassay allowing the detection and quantification of peanut allergens. A peanut specific antibody was raised targeting the major identified peanut allergens. A suggested extraction and sample handling procedure was adapted to major food products of interest with the minimum matrix interference.

Peanut DiagnoKit™ allows the immediate quantification of peanut proteins.

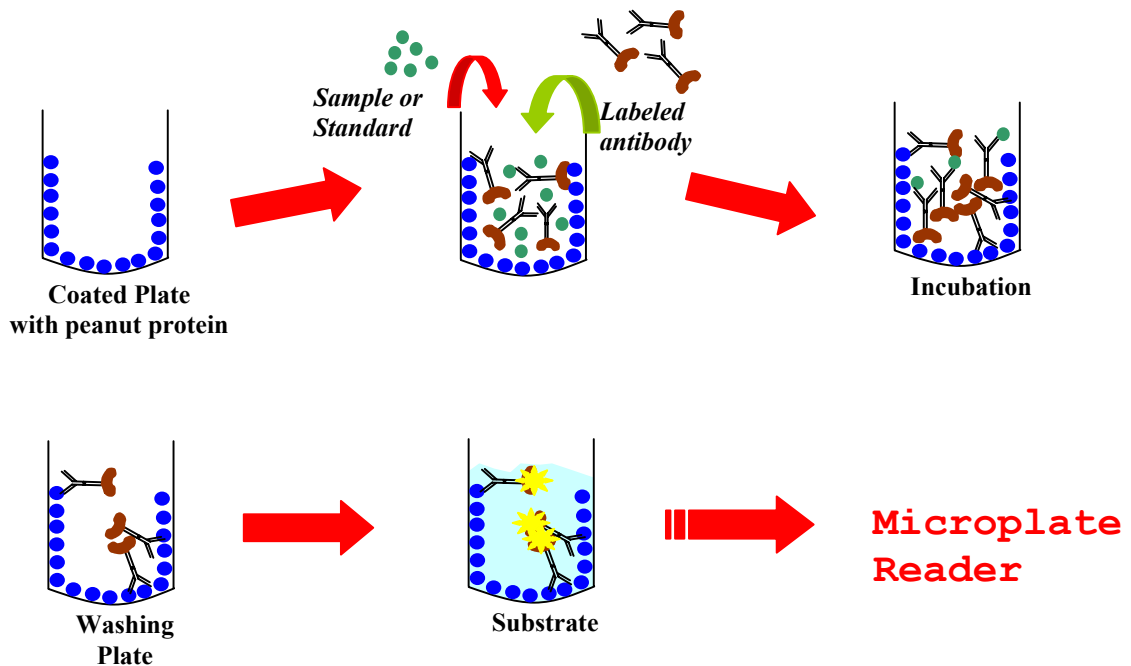


## Kit Description:

- **Direct** enzyme-immunoassay for the quantitative analysis of peanut.
- Suggested use: chocolate, cereal and ice cream samples.

## Test Principle:

The test is based on a competitive binding of Peanut labeled antibody, plate-immobilized and free peanut proteins in a standard or sample solution.



## Kit Content provided:

- Calculation diskette (MS Excel)
- 1 mL Peanut protein standard\*\* (100 µg/mL)\*
- Peanut antibody-HRP conjugate\*\* (60 µL)
- 1 microtiter plate (12 strips of 8 wells) coated with Peanut proteins.
- Dilution Buffer A (to be used for the calibration curve only) (10 mL)
- Washing buffer (dry powder)
- Dilution buffer B (dry powder)
- Substrate A, containing TMB (20 mL)
- Substrate B, containing H<sub>2</sub>O<sub>2</sub>\*\*\* (1 mL)
- Stop solution, containing H<sub>2</sub>SO<sub>4</sub>\*\*\* (10 mL)

\* Concentrations are based on a total protein amount determined by a BCA protein test

\*\*Contains Thimerosal as preservative

\*\*\*Corrosive, use with care



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Materials required, not provided:

- ❖ Precision adjustable pipets and a 12 or 8 channel multipipet able to deliver 200µl
- ❖ Plate reader with 450 nm interference filter
- ❖ Orbital shaker
- ❖ Vortex system
- ❖ Test tubes
- ❖ 500mL squeeze bottle
- ❖ De-ionized water
- ❖ Timer

Additional useful items:

- ❖ Washing buffer (cat.# D0310)
- ❖ Dilution buffer (cat.#D0320)
- ❖ Extraction buffer (cat.#D0330)
- ❖ Protein standard solution (cat #D0115)

**Safety Precautions:**

Items included in this kit are to be used by suitable qualified laboratory personnel, under proper laboratory working conditions. Handle all reagents and antibody in accordance with local safety procedures. Avoid any skin contact with stop solution and substrate B, in case of contact wash very well with water. Antibody-HRP solution contains thimerosal as preservative. Avoid contact of the reagent with the skin. MSDS (Material Safety Data Sheets) available upon request.

**Procedural notes:**

Store the kit at 2-8 °C. Before start the assay all reagents should be equilibrated at room temperature. Return all reagents to 2-8°C immediately after use. Do not interchange reagents between kits of different lot numbers. Do not use reagents beyond the expiration date of the kit. Substrate solution is light sensitive. Avoid exposure to direct light, and avoid contact with metal, which can cause colour development. A dark blue colour developed by the substrate solution after preparation is indicative of contamination. Sample extracts can be stored at 2-8°C for seven days and at -20°C for several months.



## Preparation of Reagents

*Prepare fresh diluted reagents, just prior to use*

### Washing Buffer:

Washing buffer is lyophilized and equivalent to 300 mL. Dissolve the dry powder in 30 mL of purified water for obtaining washing buffer **10 times more concentrated**; then dilute 1:10 with purified water the necessary volume.

### Dilution Buffer B:

Dilution buffer B is lyophilized and equivalent to 100 mL. Dissolve the dry powder in 100 mL of purified water. Vortex for obtaining a clear solution if necessary.

### Standard solution:

Standard solutions should be prepared immediately prior to use in suitable glassware. Standard solutions may be obtained through the following dilution scheme:

100  $\mu$ L of stock standard solution of 100  $\mu$ g/mL + 900 $\mu$ L of **dilution buffer A** to obtain 10  $\mu$ g/mL  
→**Standard 1**

Serial dilute 1 in 2 with **dilution buffer A**: 500 $\mu$ L of standard solution 1 + 500 $\mu$ L of **dilution buffer A** (1mL total) to obtain:

Standard 2 → 5  $\mu$ g/mL

Serial dilute 1 in 2 with **dilution buffer A**:

Standard 3 → 2.5 $\mu$ g/mL

Standard 4 → 1.25  $\mu$ g/mL

Standard 5 → 0.625  $\mu$ g/mL

Standard 6 → 0.313  $\mu$ g/mL

Standard 7 → 0.156  $\mu$ g/mL

### Peanut Antibody-HRP conjugate:

Dilution 1:400 of the solution provided: Pipette 30  $\mu$ L of peanut antibody-HRP conjugate and dilute to 12 mL with dilution buffer B.

### Substrate solution:

This solution should be prepared immediately prior to its use, by mixing the Substrate Solution A & B in the following proportion: 10 mL of Substrate Solution A + 5  $\mu$ L of Substrate Solution B. Prepare only the needed amount of this solution. (e.g.: for 3 strips you should prepare a total of 5 mL)

### Stopping solution:

Ready to use.

### Samples:

Samples should be diluted no less than 1:100 in Dilution buffer B in order to avoid matrix effects.



### Test Procedure

1. Prepare standards as described in **Preparation of Reagents**.
2. Rinsing of the plate: Fill each well to the top with washing buffer, using a squeeze bottle or a multichannel pipet; turn the plate upside down and empty wells. The rinsing cycle should be carried out 3 times. Remove residual liquid by tapping the plate upside down on an absorbent paper.
3. Using a precision pipet transfer 100 µL of each standard solution for calibration into a well on the plate, according with the following scheme (use 100µL of **Dilution Buffer A** in the Blank wells):

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank 0µg/mL	Blank 0µg/mL										
B	Standard 0.156µg/mL	Standard 0.156µg/mL										
C	Standard 0.313µg/mL	Standard 0.313µg/mL										
D	Standard 0.625µg/mL	Standard 0.625µg/mL										
E	Standard 1.25µg/mL	Standard 1.25µg/mL										
F	Standard 2.5µg/mL	Standard 2.5µg/mL										
G	Standard 5µg/mL	Standard 5µg/mL										
H	Standard 10µg/mL	Standard 10µg/mL										

**Sample Wells**

4. Using a precision pipet transfer 100µL of each diluted unknown sample extract into assigned well (in duplicate or triplicate).
5. Shake the plate 5 minutes on orbital shaker

***Addition of the Antibody enzyme conjugate***

6. Using a precision pipet transfer 100µL of the diluted peanut antibody-HRP conjugated solution, into each well.

***Incubation on plate***

7. Incubate the plate for 60 min. at room temperature in orbital shaker.
8. Empty the plate by inverting it over the sink then wash each well 5 times following the same protocol of step 2.
9. Add 200µL of the substrate solution A+B to each well. Mix thoroughly and incubate for 20 minutes in the dark at room temperature.
10. Add 50µL of the stop solution to each well. Mix and incubate for 10 minutes in the dark at room temperature.
11. Take measurement of the absorbance with a plate reader at 450nm.



## Results

An example of data processing is presented under a Micro Soft excel format and provided in the attached disk. A calculation table allows you to tabulate the mean O.D. for a duplicate or triplicate run or of standard solution. Resulting graph will be suggested.

Data is treated so as the mean value of the absorbance (450nm) readings obtained for the standards and the samples are reported to the absorbance value of the zero standard.

$$\left[ \frac{\text{Absorbance standard (or sample)}}{\text{Absorbance zero standard}} \right] \times 100 = \% \text{ B/Bo}$$

Maximum OD Blank = zero standard

A calibration curve can be obtained using the calculated % B/Bo value for each standard vs the log of the corresponding Protein concentration (in ng/ml).

Take the B/Bo (%) value for each sample and interpolate the corresponding concentration from the calibration curve. The linear transformation of this calibration curve may be obtained by plotting, logit (%B/Bo) vs ln C where:

$$\text{logit \% B/Bo} = \ln \left[ \frac{\% \text{B/Bo}}{100 - \% \text{B/Bo}} \right]$$

➤ see provided disk, MS-Excel file

In order to obtain the unknown concentration in ng/ml contained in a sample, use one of the linear ranges of the calibration curve of your choice. The determined value must be further multiplied by the corresponding dilution factor. This is based on the assumption that the recovery after extraction is 100%.

**Positives may be considered certain when the O.D. obtained for a sample is 15% lower than that of the blank solution of the calibration curves. As matrix effect may appear, dilutions of sample may be beneficial. (A 1:100 dilution is suggested for the performance of this test on chocolate extracts). The standard diluent (Buffer A) was specially designed to mimic most commonly encountered matrices.**



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## **Suggested extraction for chocolate**

### **Buffers:**

**PBS:** Phosphate Buffer Saline (PBS adjusted to 7.4) contained 20 mmol of  $\text{NaH}_2\text{PO}_4$  and 140 mmol of NaCl per liter of de-ionized water.

**Extraction Buffer** contained 1.0 M NaCl, 0.1 M PBS, 0.1% (w/v) Tween 20, 0.5% Triton x-100, pH 7.0)

### **Material and Method:**

1. Weigh out 10.0 g of sample into 250 mL screw top centrifuge tube.
2. Break up sample into smaller pieces.
3. Add 100mL extraction buffer to each sample (100 mL for 10.0 g sample).
4. Shake samples vigorously for one hour in a heated water bath set at 45 °C.
5. Centrifuge each sample at 3,000 rpm for 5 minutes.
6. Remove supernatant
7. Centrifuge supernatant for 30 minutes at 20,000 g in a refrigerated centrifuge at 4°C.
8. Filter the extract through Whatman # 1 filter paper and refrigerate.

Extracts will remain stable over a period of seven days or can be stored at  $-20^\circ\text{C}$  for longer periods.

### **TECHNICAL SUPPORT:**

#### ***Abkem Iberia S.L.***

Tomás A. Alonso 160, 2º

36208 Vigo - SPAIN

**Tel:** +34-986 24 70 83

**Fax:** +34-986 24 70 84

**E-mail:** [info@abkemiberia.com](mailto:info@abkemiberia.com)

[techsupport@abkemiberia.com](mailto:techsupport@abkemiberia.com)