7.0 Appendix

1. Kit Protocols

1.1 Ridascreen® Gliadin

RIDASCREEN® Gliadin

Enzymimmunoassay zur quantitativen Bestimmung von Gliadinen und verwandten Prolaminen

Enzyme immunoassay for the quantitative analysis of gliadins and corresponding prolamines

Art. No.: R7001

PERFORMANCE TESTED
AOAC
RESEARCH INSTITUTE
LICENSE NUMBER 120601

In vitro Test
Lagerung bei 2 - 8 °C
Storage at 2 - 8 °C

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RIDASCREEN® Gliadin

Brief information

RIDASCREEN® Gliadin (Art. No. R7001) is a sandwich enzyme immunoassay for the quantitative analysis of prolamins from wheat (gliadin), rye (secalin) and barley (hordein) in as gluten-free declared food.
The RIDASCREEN® Gliadin test has been validated and certified with the license no. 120601 as a Performance Tested Method by the AOAC Research Institute. RIDASCREEN® Gliadin is also the official method (type 1) of the Codex Alimentarius.
All reagents required for the enzyme immunoassay - including standards - are contained in the test kit. The test kit is sufficient for 96 determinations (including standards). A microtiter plate spectrophotometer is required for quantification.

Sample preparation:	homogenization and extraction
Standard material:	The RIDASCREEN® standard material is calibrated to the standard of the Prolamin Working Group.
Time requirement:	sample preparation (for 10 samples) .....approx. 2 h test implementation (incubation time) .....1.5 h
Detection limit:	1.5 ppm gliadin corresponding to 3 ppm gluten
Limit of quantification:	2.5 ppm gliadin corresponding to 5 ppm gluten
Recovery rate:	92 - 113 % for different food matrices
Specificity:	The monoclonal antibody R5 reacts with the gliadin-fractions from wheat and corresponding prolamins from rye and barley.
Cross reactivity:	No cross reaction with soy, oats, corn (maize), rice, millet, teff, buckwheat, quinoa and amaranth.

Related products
RIDASCREEN®FAST Gliadin (Art. Nr.: R7002)
RIDASCREEN®QUICK Gliadin (Art. Nr.: R7003)
RIDASCREEN® Gliadin competitive (Art. Nr.: R7011)
Cocktail Solution (R7006) or RIDA® Extraction Solution (R7099)
Set of 3 Gliadin Assay Controls (Art. Nr.: R7010)
SureFood® Allergen real time PCR Gluten (S3106)

1. Intended use

RIDASCREEN® Gliadin is a sandwich enzyme immunoassay for the quantitative analysis of contaminations by prolamins from wheat (gliadin), rye (secalin), and barley (hordein) in raw products like flours (buckwheat, rice, corn, oats, teff) and spices as well as in processed food like noodles, ready-to-serve meals, bakery products, sausages, beverages and ice cream.
All samples should be extracted with the Cocktail Solution (Art. No. R7006, official R5-Mendez method) or the RIDA® Extraction Solution (Art. No. R7099). Further application notes are available at R-Biopharm.

2. General

The use of wheat flour and gluten in foodstuffs is extremely common because of their heat stability and useful effects on e.g. texture, moisture retention and flavour. Gluten is a mixture of prolamin and glutelin proteins present in wheat, rye and barley.

Coeliac disease is a permanent intolerance to gluten that results in damage to the small intestine and is reversible when gluten is avoided by diet.

According to the Codex Alimentarius (Alinorm 08/31/26) two categories for labeling of food according to the gluten content now exist:
1. Food products which contain less than 20 ppm can be labeled as **"gluten-free"**.
2. Food products labeled as **"very low gluten"** can have a gluten content above 20 and up to 100 ppm.

3. Test principle

The basis of the test is the antigen-antibody reaction. The wells of the microtiter strips are coated with specific antibodies against gliadins. By adding the standard or sample solution to the wells, present gliadin will bind to the specific capture antibodies. The result is an antibody-antigen-complex. Components not bound by the antibodies are then removed in a washing step. Then antibody conjugated to peroxidase is added. This antibody-conjugate is bound to the Ab-Ag-complex. An antibody-antigen-antibody (sandwich) complex is formed. Any unbound enzyme conjugate is then removed in a washing step. Enzyme substrate and chromogen are added to the wells and incubated. Bound enzyme conjugate converts the colorless chromogen into a blue product. The addition of the stop reagent leads to a color change from blue to yellow. The measurement is made photometrically at 450 nm. The absorbance is proportional to the gliadin concentration of the sample.

4. Reagents provided

Each kit contains sufficient materials for 96 measurements (including standard analyses). Each test kit contains:

1 x Microtiter plate (12 strips with 8 removable wells each)
coated with antibodies against gliadin
6 x Gliadin Standards, 1.3 ml each
0 ppb (zero standard), 5 ppb, 10 ppb, 20 ppb, 40 ppb, 80 ppb gliadin
in aqueous solution, ready-to-use
1 x Conjugate (1.2 ml) ..... red cap
peroxidase conjugated antibody, concentrate
1 x Substrate (7 ml) ..... green cap
contains urea peroxide
1 x Chromogen (7 ml) ..... blue cap
contains tetramethylbenzidine
1 x Stop solution (14 ml) ..... yellow cap
contains 1 N sulfuric acid
1 x Sample diluent (60 ml) ..... white cap
as a 5x concentrate
1 x Washing buffer (100 ml) ..... brown cap
10x concentrate

5. Reagents required but not provided

5.1. Equipment:

– microtiter plate spectrophotometer (450 nm)
– centrifuge + centrifugal glass vials
– shaker
– laboratory mincer / grinder, pestle and mortar, ultra-turrax or homogenizer
– water bath (50 °C / 122 °F)
– graduated pipettes
– variable 20 µl - 200 µl and 200 - 1000 µl micropipettes

5.2. Reagents:

– distilled or deionized water
– skim milk powder (food quality)

– Cocktail Solution, Art. No. R7006, (105 ml) for sample preparation

– ethanol solution (80 %), for the sample extraction with Cocktail Solution: add 120 ml ethanol p.a. to 30 ml distilled water and shake well

or

– RIDA® Extraction Solution, Art. No. R7099, (105 ml) for sample preparation (9.6.)

– 2-propanol solution (68 %), for the sample extraction with RIDA® Extraction Solution: add 68 ml 2-propanol p.a. to 32 ml distilled water and shake well

6. Warnings and precautions for the users

The stop solution contains 1 N sulfuric acid (R36/38, S2-26).

The buffer contains thimerosal. Avoid contact of the reagent with the skin.

7. Storage instructions

Store the kit at 2 - 8 °C (35 - 46 °F). Do not freeze any test kit components.

Return any unused microwells to their original foil bag, reseal them together with the desiccant provided and further store at 2 - 8 °C (35 - 46 °F).

The colorless chromogen is light sensitive, therefore, avoid exposure to direct light.

No quality guarantee is accepted after expiry of the kit (see kit label).

Do not interchange individual reagents between kits of different lot numbers.

8. Indication of instability or deterioration of reagents

–any coloration of the chromogen solution prior to test implementation
–a value of less than 0.6 absorbance units ($A_{450\ nm} < 0.6$) for standard 6

9. Preparation of Samples

9.1. Preliminary comments

Airborne cereal dust and dirty laboratory equipment lead to gliadin contamination of the assay. Therefore, wear gloves during the assay and before starting with the assay:

- clean surfaces, glass vials, mincers and other equipment with 80 % ethanol or 2-propanol (see 5.2.)
- carry out the sample preparation in a room isolated from the ELISA procedure
- check for gliadin contamination of reagents and equipment with the test strips RIDA®QUICK Gliadin (Art. No. R7003)
- it is recommended to work **under a chemical hood,** because of ß-mercapto-ethanol in the Cocktail Solution and the RIDA® Extraction Solution
- ß-mercaptoethanol can disturb the ELISA, therefore dilute the samples **at least 1:500** (recommendation 1:500 for samples with approx. 20 mg/kg gluten and 1:2500 for samples with approx. 100 mg/kg gluten).

9.2. Extraction with the cocktail solution (Art. No. R7006, official AOAC-RI method)

Homogenize well a sufficient amount (at least 5 g or 5 ml) of sample (grind it thoroughly to powder and mix well or mix well the solution respectively).

- **liquid food samples**: use 0.25 ml of the homogenized sample and add 2.5 ml of the Cocktail Solution, close the vial and mix well
- **other food samples (e.g. soy and millet containing samples)**: weigh 0.25 g of the homogenized sample and add 2.5 ml of the Cocktail Solution, close the vial and mix well
- **tannin and polyphenol containing food samples (e.g. chocolate, coffee, cocoa, chestnut flour, quinoa and buckwheat)**: weigh 0.25 g of the homogenized sample, add 0.25 g of skimmed milk powder and add 2.5 ml of the Cocktail Solution, close the vial and mix well
- **meat and sausages:** in these matrices gliadin may be not distributed evenly, therefore, weigh 50 g sample and homogenize
- weigh 0.25 g of the homogenized sample and add 2.5 ml of the Cocktail Solution, close the vial and mix well

Please further extract all samples as described in the following:

- incubate for 40 min at 50 °C (122 °F)
- let the sample cool down and then mix it with 7.5 ml 80 % ethanol (see 5.2.)
- close the vial and shake for 1 h up side down or by a rotator at room temperature (20 - 25 °C / 68 - 77 °F)
- centrifuge: 10 min / at least 2500 g / room temperature (20 - 25 °C / 68 - 77 °F)
- put the supernatant in a screw top vial
- dilute the sample 1:12.5 (1+11.5 / 80 µl + 920 µl) with diluted sample diluent (see 10.1.): the final dilution factor is 500
- use 100 µl per well in the assay

9.3. Extraction with the RIDA® Extraction solution (Art. No. R7099)

The extraction with the RIDA® Extraction solution is more timesaving than with cocktail solution.

Homogenize well a sufficient amount (at least 5 g or 5 ml) of sample (grind it thoroughly to powder and mix well or mix well the solution respectively).

- **liquid food samples**: use 0.25 ml of the homogenized sample and add 2.5 ml of the RIDA® Extraction Solution, close the vial and mix well
- **other food samples**: weigh 0.25 g of the homogenized sample and add 2.5 ml of the RIDA® Extraction Solution, close the vial and mix well
- **soy, millet, quinoa or buckwheat containing food samples as well as further tannin and polyphenol containing food samples (e.g. chocolate, coffee or cocoa, chestnut flour)**: weigh 0.25 g of the homogenized sample, add 0.25 g of skimmed milk powder and add 2.5 ml of the RIDA® Extraction Solution, close the vial and mix well
- **meat and sausages:** in these matrices gliadin may be not distributed evenly, therefore, weigh 50 g sample and homogenize
- weigh 0.25 g of the homogenized sample and add 2.5 ml of the RIDA® Extraction Solution, close the vial and mix well

Please further extract all samples as described in the following:

- incubate for 15 min at 60 °C (122 °F)
- let the sample cool down and then mix it with 7.5 ml 68 % 2-propanol (see 5.2.)
- close the vial and incubate for 10 min in a water bath at 60 °C (140 °F)
- centrifuge: 10 min / at least 2500 g / room temperature (20 - 25 °C / 68 - 77 °F)

– put the supernatant in a screw top vial
– dilute the sample 1:12.5 (1+11.5 / 80 µl + 920 µl) with diluted sample diluent (see 10.1.): the final dilution factor is 500
– use 100 µl per well in the assay

Remark:

All supernatants obtained after the centrifugation can be stored in a tightly closed vial in the dark at room temperature (20 - 25 °C / 68 - 77 °F) up to eight weeks.

10. Test implementation

10.1. Test preparation

Bring all reagents to room temperature (20 - 25 °C / 68 - 77 °F) before use.

The **sample diluent** is provided as a concentrate (5fold). Only the amount which actually is needed should be diluted 1:5 (1+4) with distilled water (e. g. 3 ml concentrate + 12 ml distilled water, sufficient for the dilution of 10 samples). Make sure that the buffer is not contaminated with gliadin.

The **antibody enzyme conjugate** (bottle with red cap) is provided as a concentrate (11fold). Since the diluted enzyme conjugate solution has a limited stability, only the amount which actually is needed should be diluted. Before pipetting, the conjugate concentrate should be shaken carefully. For reconstitution, the conjugate concentrate is diluted 1:11 (1+10) with distilled water (e. g. 200 µl concentrate + 2 ml distilled water, sufficient for 2 microtiter strips). Take care that the water is not contaminated with gliadin.

The **washing buffer** is provided as a 10fold concentrate. Before use, the buffer has to be diluted 1:10 (1+9) with distilled water (i.e. 100 ml buffer concentrate + 900 ml dist. water). Prior to dilution, dissolve eventually formed crystals by incubating the buffer in a water bath at 37 °C (99 °F). The diluted buffer is stable at 2 - 8 °C (35 - 46 °F) for four weeks.

10.2. Test procedure

Carefully follow the recommended washing procedure. Do not allow microwells to dry between working steps.

1. Insert a sufficient number of wells into the microwell holder for all standards and samples to be run in duplicate. Record standard and sample positions.
2. Add 100 µl of each standard solution or sample to separate duplicate wells and incubate for 30 min at room temperature (20 - 25 °C / 68 - 77 °F).
3. Pour the liquid out of the wells and tap the microwell holder upside down vigorously (three times) against absorbent paper to ensure complete removal of liquid from the wells. Fill all the wells with 250 µl diluted washing buffer (see 10.1.) and pour out the liquid again. Repeat two more times.
4. Add 100 µl of the diluted enzyme conjugate (see 10.1.) to each well and incubate for 30 min at room temperature (20 - 25 °C / 68 - 77 °F).
5. Pour the liquid out of the wells and tap the microwell holder upside down vigorously (three times in a row) against absorbent paper to ensure complete removal of liquid from the wells. Fill all the wells with 250 µl diluted washing buffer (see 10.1.) and pour out the liquid again. Repeat two more times.
6. Add 50 µl of substrate and 50 µl of chromogen to each well. Mix gently by skaking the plate manually and incubate for 30 min at room temperature (20 - 25 °C / 68 - 77 °F) in the dark.
7. Add 100 µl of the stop reagent to each well. Mix gently by skaking the plate manually and measure the absorbance at 450 nm. Read within 30 min after addition of stop solution.

11. Results

A special software, the RIDA®SOFT Win (Art. No. Z9999), is available for evaluation of the RIDASCREEN® enzyme immunoassays. The calculation should be done by use of a cubic spline function.

The course of the standard curve is shown in the Quality Assurance Certificate enclosed in the test kit. For quality assurance Gliadin Assay Controls (Art. Nr. R7010) should be used.

In comparison with the certificate, higher values of the absorbance ($A_{450\ nm}$) for the calibration curve, especially for the zero standard, may be a result of insufficient washing or gliadin contamination.
A further dilution and new detection of the samples is recommended for absorbance values ($A_{450\ nm}$) > standard 6. The samples should be diluted so that the results can be read in the linear part of the calibration curve.

The gliadin concentration in µg/kg (ppb) is read from the calibration curve and must be further multiplied by the recommended dilution factor of 500. This result is then multiplied by 2 in order to obtain the gluten concentration (gliadin usually represents 50 % of the proteins present in gluten).

Example:
The absorbance value of a sample corresponds to 10 µg/kg gliadin in the calibration curve. Multiplying by the recommended dilution factor 500 leads to 5000 µg/kg, corresponding to 5 mg/kg (ppm) gliadin, respectively 0.0005 % gliadin. To calculate the gluten content, it is necessary to multiply by factor 2 which results in 10 ppm gluten, respectively 0.001 % gluten. This sample is considered to be gluten-free, because the gluten concentration is below 20 ppm.

The product information folder with further information and the comparison between the extraction with cocktail solution and with the RIDA® Extraction solution is available on request from your local distributor or R-Biopharm AG.

1.2 Ridascreen® FAST Milk

RIDASCREEN®FAST Milk

Brief information

RIDASCREEN®FAST Milk (Art. No.: R4652) is a sandwich enzyme immunoassay for the quantitative analysis of milk protein in food.
All reagents required for the enzyme immunoassay - including standards - are contained in the test kit.
The test kit is sufficient for 48 determinations (including standards).
A microtiter plate spectrophotometer is required for quantification.

Sample preparation:	homogenization, extraction and centrifugation
Time requirement:	sample preparation..... approx. 45 min test implementation (incubation time)..... 30 min
Limit of detection:	0.7 mg/kg (ppm) milk protein
Limit of quantification:	2.5 mg/kg (ppm) milk protein
Standard material:	The RIDASCREEN® standard material is calibrated to the reference material NIST RM 1549 skim milk powder.
Specificity:	The antibodies specifically detect α-, ß- and κ-caseins as well as ß-lactoglobulin of cow's milk and of sheep's, goat's and buffalo's milk.

1. Intended use

RIDASCREEN®FAST Milk is a sandwich enzyme immunoassay for the quantitative analysis of milk protein in food which may contain whey, milk or milk powder like sausages, ice cream, chocolate, bakery goods, cake and bread mix, soups, sauces, dressings and beverages (juice, wine, beer).

2. General

Cow`s milk contains 3.2 % proteins which consist of 10 % ß-lactoglobulin (leading protein of whey) and 80 % caseins. The most important allergen is ß-lactoglobulin especially for children while the caseins become to be dominant later in adults.

Casein (lat. Caseus = cheese) is a rough flaked curdling protein, which forms micells in the milk and precipitates under acidic conditions. The group of caseins consists of the α_s-caseins, the ß-caseins, the κ-casein, and the γ-caseins (proteolytic protein fragment of ß-casein by the milk protease plasmin). κ-casein is cleaved into a hydrophobic (para κ-casein) and into a water soluble polar component (macropeptid) by proteolysis, e.g. by using lab ferment.
The main components of whey are ß-lactoglobulin and alpha-lactalbumin besides lactoferrin, bovine serum albumin and immunoglobulins.

Milk can be present as an ingredient or as a contamination in raw and processed food products. Because milk can induce allergic reactions at infancy it must be declared as an ingredient on food labels. Whey (ß-lactoglobulin) or caseinates (e.g. in sausages) are often added to food products, therefore it is recommended to determine casein in food.

3. Test principle

The basis of the test is the antigen-antibody reaction. The wells of the microtiter strips are coated with specific antibodies against the milk proteins casein and ß-lactoglobulin. By adding the standard or sample solution to the wells, present milk protein will bind to the specific antibodies. The result is an antibody-antigen-complex.
In a washing step components not bound are removed. Then antibody conjugated to peroxidase (enzyme conjugate) is added. This antibody conjugate is bound to the antibody-antigen-complex. An antibody-antigen-antibody-complex (sandwich) is formed. Substrate/chromogen is added after removing of any unbound enzyme conjugate in a washing step. Bound enzyme conjugate converts the chromogen into a blue product.

The addition of the stop solution leads to a color change from blue to yellow. The measurement is made photometrically at 450 nm. The absorption is proportional to the milk protein concentration in the sample.

4. Reagents provided

Each kit contains sufficient materials for 48 measurements (including standard analyses). Each test kit contains:

1 x Microtiter plate with 48 wells (6 strips with 8 removable wells each)
coated with anti-casein and anti-ß-lactoglobulin antibodies
5 x Standards *), 1.3 ml each
0 ppm (zero standard), 2.5 ppm, 7.5 ppm, 22.5 ppm, 67.5 ppm milk protein
in aqueous solution, ready to use
1 x Conjugate (0.7 ml) ..... red cap
peroxidase conjugated antibody, concentrate
1 x Conjugate dilution buffer (7 ml) ..... black cap
ready to use
1 x Red Chromogen Pro (10 ml) ..... brown cap
substrate/chromogen, stained red
1 x Stop solution (14 ml) ..... yellow cap
contains 1 N sulfuric acid
1 x Allergen Extraction buffer (125 ml) ..... green cap
as a **10fold concentrate**
1 x Extraction Solution 2 (110 ml) ..... blue cap
as a 2fold concentrate
1 x Wash buffer (100 ml) ..... brown cap
as a 10fold concentrate
1 x Additive 1 (2 g) ..... blue cap

*) **The dilution factor 100** which results after sample preparation has already been considered for the standard concentrations. Therefore, the milk protein concentrations of the samples can directly be read from the standard curve.

5. Reagents required but not provided

5.1. Equipment:

–microtiter plate spectrophotometer (450 nm)
–centrifugal vials
–water bath (60 °C / 140 °F and 100 °C / 212 °F)

–fluted paper filter
–variable 20 - 200 µl and 200 - 1000 µl micropipettes

5.2. Reagents:

–distilled water
–1 M hydrochloric acid (HCl)
–1 M sodium hydroxide (NaOH)

6. Warnings and precautions for the users

This test should only be carried out by trained laboratory employees. The instruction for use must be strictly followed.

The washing buffer contains thimerosal. Avoid skin contact.

The stop solution contains 1 N sulfuric acid (R36/38, S2-26).

The Extraction Solution 2 contains ß-mercaptoethanol and should be handled carefully under a chemical hood.

7. Storage instructions

Store the kit at 2 - 8 °C (35 - 46 °F). Do not freeze any test kit components.

Return any unused microwells to their original foil bag, reseal them together with the desiccant provided and further store at 2 - 8 °C (35 - 46 °F).

The substrate/chromogen solution is light sensitive, therefore, avoid exposure to direct light.

No quality guarantee is accepted after the expiration date on the kit label.

Do not interchange individual reagents between kits of different lot numbers.

8. Indication of instability or deterioration of reagents

–any bluish coloration of the red stained substrate/chromogen solution prior to test implementation
–a value of less than 0.8 absorbance units ($A_{450\ nm} < 0.8$) for standard 5

9. Preparation of samples

9.1. Preparations

Bring all reagents to room temperature (20 - 25 °C / 68 - 77 °F) before use.

The **Allergen Extraction buffer** is provided as a **10fold concentrate**. Before dilution dissolve possibly formed crystals by heating (water bath 37 °C) and mix well. Dilute the heated concentrate 1:10 (1+9) with distilled water (e.g. 100 ml concentrate + 900 ml dist. water) or fill up the whole content of the bottle to 1250 ml. The diluted Allergen Extraction buffer can be used for approx. 12 weeks at 2 - 8 °C.

For the preparation of the **Allergen Extraction buffer, containing Additive 1 (A-AEP)** weigh 1.8 g of Additive 1 in a glass beaker and add 20 ml 1 M NaOH. Stir until the Additive 1 is solved. Then fill 900 ml diluted Allergen Extraction buffer (see above) in a measuring cylinder. Add the 20 ml Additive 1 solution by stirring constantly, transfer eventually residues of the Additive 1 solution into the measuring cylinder by rinsing with diluted Allergen Extraction buffer. Adjust the Additive 1 containing Allergen Extraction (A-AEP) buffer to pH 9 with 1 M HCl and fill up to 1 L with diluted Allergen Extraction buffer.
1 L A-AEP buffer is sufficient for 60 samples. The buffer can be used for approx. 3 weeks at room temperature (20 - 25 °C) or for 8 weeks at 2 - 8 °C (if crystals precipitate the buffer must be discarded).

The **Extraction solution 2** is provided as a 2fold concentrate and has to be diluted 1:2 (1+1) with distilled water (e.g. 50 ml Extraction solution 2 + 50 ml dist. water). The complete diluted Extraction solution 2 is sufficient for 55 samples and can be used for approx. 3 month at room temperature (20 - 25 °C).

9.2. Sample extraction

–homogenize a representative amount of the sample (5 - 50 g)

Solid samples:

–take 1 g and add 4 ml prepared Extraction solution 2 (see 9.1.), mix vigorously, close the vial and cook it for 10 min at 100 °C in a water bath
–let the sample cool down shortly
–add 16 ml heated (60 °C) A-AEP (see 9.1.) to the cooked sample

Liquid samples:

- take 1 ml and add 4 ml prepared Extraction solution 2 (see 9.1.), mix vigorously, close the vial and cook it for 10 min at 100 °C in a water bath
- let the sample cool down shortly
- add 15 ml heated (60 °C) A-AEP to the cooked sample

further prepare solid and liquid samples as follows:

- mix vigorously (shaker)
- extract for 10 min at 60 °C in a water bath
- cool down (e.g. ice water), centrifuge for 10 min / 2500 g (alternatively: 2 ml of the extract can be centrifuged with high speed for 10 min in reaction caps by using a microcentrifuge)
- filter the supernatant (with fluted paper filter)
- dilute the sample 1:5 (1+4) with final diluted Allergen Extraction buffer, without Additive 1 (see 9.1.), e.g. 100 µl + 400 µl (1:100 final) (**Remark:** use this diluted supernatant directly in the assay)
- use 100 µl per well in the assay

The sample extracts can be stored at 2 - 8 °C (35 - 46 °F) for 3 days. The extracts can be stored at -20 °C (-4 °F) for several months.

10. Test implementation

10.1. Test preparation for the ELISA

Bring all reagents to room temperature (20 - 25 °C / 68 - 77 °F) before use.

The **antibody enzyme conjugate** (bottle with red cap) is provided as a 11fold concentrate. Since the diluted enzyme conjugate solution has a limited stability, only the amount which actually is needed should be diluted. Before pipetting, the conjugate concentrate should be shaken carefully. For reconstitution, the conjugate concentrate is diluted 1:11 (1+10) with conjugate dilution buffer (e. g. 200 µl concentrate + 2 ml buffer, sufficient for 2 microtiter strips).

The **washing buffer** is provided as a 10fold concentrate. Before use, the buffer has to be diluted 1:10 (1+9) with distilled water (i.e. 100 ml buffer concentrate + 900 ml dist. water). Prior to dilution, dissolve eventually formed crystals by incubating the buffer in a water bath at 37 °C (99 °F). The diluted buffer is stable at 2 - 8 °C (35 - 46 °F) for four weeks.

10.2. Test procedure

Carefully follow the recommended washing procedure. Do not allow microwells to dry between working steps.
Do not use more than three strips (24 wells) at a time.

1. Insert a sufficient number of wells into the microwell holder for all standards and samples to be run in duplicate. Record standard and sample positions.
2. Add 100 µl of each standard solution or prepared sample to separate duplicate wells and incubate for 10 min at room temperature (20 - 25 °C / 68 - 77 °F).
3. Pour the liquid out of the wells and tap the microwell holder upside down vigorously (three times in a row) against absorbent paper to ensure complete removal of liquid from the wells. Fill all the wells with 250 µl washing buffer (see 10.1.) and pour out the liquid again. Repeat three more times.
4. Add 100 µl of the diluted enzyme conjugate to each well. Mix gently by shaking the plate manually and incubate for 10 min at room temperature (20 - 25 °C / 68 -77 °F).
5. Pour the liquid out of the wells and tap the microwell holder upside down vigorously (three times in a row) against absorbent paper to ensure complete removal of liquid from the wells. Fill all the wells with 250 µl washing buffer (see 10.1.) and pour out the liquid again. Repeat three more times.
6. Add 100 µl of the reddish Substrate/Chromogen solution to each well. Mix gently by shaking the plate manually and incubate for 10 min at room temperature (20 - 25 °C / 68 - 77 °F) in the dark.
7. Add 100 µl of the stop solution to each well. Mix gently by shaking the plate manually and measure the absorbance at 450 nm. Read within 10 minutes after addition of stop solution.

11. Results

A special software, the RIDA®SOFT Win (Art. No. Z9999), is available for evaluation of the RIDASCREEN® enzyme immunoassays. The calculation should be done by use of a cubic spline function.

The course of the standard curve is shown in the Quality Assurance Certificate enclosed in the test kit.

In comparison with the certificate, higher values of the absorbance ($A_{450\ nm}$) for the calibration curve, especially for the zero standard, may be a result of insufficient washing or milk protein contamination.

A further dilution and new detection of the samples is recommended for absorbance values ($A_{450\ nm}$) > standard 5. The samples should be diluted so that the results can be read in the linear part of the calibration curve.

Please note:

When working in accordance with the regulation stated, the dilution factor is 100. The milk protein concentration can be read directly from the standard curve - the sample dilution factor of 100 is already taken into account for the standard concentrations (see 4. *).

For sample dilutions of more than 1:100 the further dilution factor must be considered for the calculation of the milk protein concentration.

Remarks:

If the result is below the detection limit, further milk constituents e.g. lactose may be present in the food sample.

Milk contains approx. 3.2 % milk protein. Thus a sample which contains 1 ppm milk protein corresponds to a milk content of approx. 31 ppm milk.

Recommendation:

In order to ensure a high analytical performance:

- each sample material should be analyzed in duplicates
- use also milk protein free and milk protein containing (spiked) samples as test controls

1.3 ELISA Systems Hazelnut Residue

Hazelnut Residue

Product Code: ESHRD-48

Microwell ELISA For Laboratory Use Only. Store Between 2 - 8°C

For screening for the presence of hazelnut protein residue in food products and environmental samples.

Directions For Use

Intended Use

The ELISA SYSTEMS Hazelnut Residue assay is an enzyme-linked immunosorbent assay (ELISA) that may be used to screen food products for the presence of hazelnut material. This assay is a rapid and reliable test that significantly reduces the time required to screen food products for the presence of hazelnut residues.

Background

The Hazelnut (*Corylus avellana*) belongs to a group of foods, commonly referred to as "tree nuts". This group of foods is a significant source of allergens and hazelnuts are a common cause of food allergy. Allergic reactions to hazelnuts range from oral allergy syndrome to severe anaphylactic reactions.[1] Tree nut material may occur unintentionally in foods due to several reasons, including: cross contamination of ingredients, food preparation errors and improper cleaning of equipment. This may lead to their presence not being listed on the ingredients label and the possibility of a product recall.

Consequently, there is a need for a sensitive and reliable test for the detection of specific tree nut allergens in foods. Any such test must be able to detect a heat-stable protein component, otherwise false negative results could occur with samples that have been heat treated.

A specific hazelnut protein was identified to be heat-stable and is used as the hazelnut protein indicator for the ELISA SYSTEMS Hazelnut Residue ELISA.

Please note: A special extraction solution is required for samples containing Polyphenols, including Dark Chocolate, Wine, Fruit Juices, Herbs, and Tannins. (Product Code: ESADDSOL).

Principle of Procedure

The ELISA SYSTEMS Hazelnut Residue ELISA is a double antibody (sandwich) ELISA utilizir specific antibodies coated onto microwells. After addition of the sample, the Enzyme Conjuga then the TMB Substrate, a positive reaction (indicating the presence of hazelnut) produces a blue colour. Addition of the Stop Solution ends the assay and turns the blue colour to yellow. The results may be read visually or with an ELISA reader.

The variation of hazelnut protein levels in different strains must be taken into consideration wh assessing the potential total hazelnut protein concentration and the allergenic issues associated with the sample being tested. The results of the testing are only applicable to the portion of the sample product tested and to this extent, ELISA SYSTEMS cannot guarantee th hazelnut material is, or is not, present in the untested portions of the sample product.

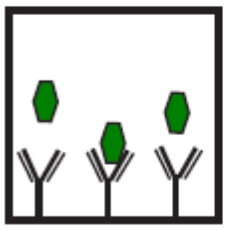
The assay is designed for screening purposes.
Any sample returning a positive result should be regarded as a presumptive result and confirmation or further testing should be performed.

Please note: A special extraction solution is required for samples containing Polyphenols, such as Dark Chocolate, Wine, Fruit Juices, Herbs, and Tannins.
One bottle of 20x Concentrate is supplied with this kit. (Product code: ESADDSOL).

How the ELISA SYSTEMS Hazelnut Residue test works:

Step 1

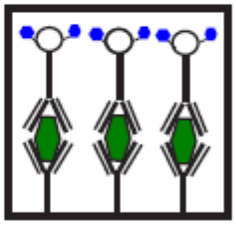
Sample is added



The test sample is added and if hazelnut residue is present, it will bind to the specific antibodies.

Step 2

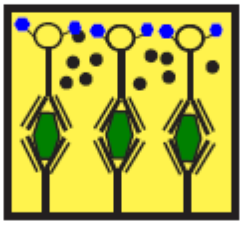
Antigen-Antibody Complex



Enzyme-labelled Conjugate is added and binds to the captured hazelnut residue to form a "sandwich".

Step 3

Coloured End-Point



TMB Substrate is added, which is converted in the presence of the Enzyme Conjugate to form a blue colour if hazelnut residue is present in the sample.

A yellow colour is formed once Acid is added to stop the reaction.

Reagents Supplied

Test Strips: microwells coated with anti-Hazelnut antibodies – 48 wells.
Test strip holder: One (1)

Negative Standard: One (1) vial containing 1.7mL of a buffered base.

Positive Standards:
One (1) vial containing 1.7mL of Hazelnut Protein in a buffer to provide a Control value of 0.5 ppm
One (1) vial containing 1.7mL of Hazelnut Protein in a buffer to provide a Control value of 1.0 ppm
One (1) vial containing 1.7mL of Hazelnut Protein in a buffer to provide a Control value of 2.5 ppm
One (1) vial containing 1.7mL of Hazelnut Protein in a buffer to provide a Control value of 5.0 ppm

Enzyme Conjugate:
One (1) bottle containing 7mL of Peroxidase-conjugated anti-Hazelnut protein polyclonal antibodies with preservative.
Please note - this reagent is light-sensitive. Avoid unnecssary exposure of the reagent to light.

Substrate: One (1) bottle containing 7mL of a stabilized Tetramethylbenzidine (TMB).
Please note - this reagent is light-sensitive. Avoid unnecssary exposure of the reagent to light.

Wash Buffer Solution concentrate (20x): Three (3) bottles containing 25mL each of concentrated wash buffer solution with preservative.

Extraction Solution concentrate (20x): Three (3) bottles containing 25mL each of concentrated extraction solution with preservative.

Extraction Solution concentrate (20x) for samples containing Polyphenols: (ESADDSOL)
One (1) bottle containing 20x Enhanced Extraction Solution concentrate.

Stop Solution: One (1) bottle containing 7mL of 1M Phosphoric acid.
(CAUTION THIS SOLUTION IS ACIDIC) Avoid contact of this solution with eyes and skin.
In case of skin contact, wash immediately with copious amounts of water. A mild soap should be used.
In case of eye contact, flush generously for at least 15 minutes with water.
Seek urgent medical attention if the irritation persists or is severe.

Additional Materials Required:

•Suitable clean containers for use in the sample extraction procedure. Do not use Polystyrene containers as these could absorb protein from the extract. We suggest low-binding capacity plastic disposable containers.
•Clean test tubes or small microtubes for aliquotting the Enzyme Conjugate and Substrate volumes prior to use.
•Data record sheets. •Fine-tipped marking-pen. •Laboratory Timer. •Paper towels.
•Plastic wash bottle with a fine tip. •Distilled or Deionized water. •Laboratory Vortex machine. •Laboratory balance capable of measuring at least 2 decimal places.
•Pipettes: 20-200 microlitre; 100-1000 microlitre (optional, for aliquoting reagents); 20-200 microlitre multichannel pipette if using more than two strips per run; disposable tips.
•Water Bath, capable of heating and holding the samples at 60°C during extraction. •Blender, Grinder, Stomacher, Ultraturrax or similar devices for sample preparation. •Disinfecting Solution or a system for Biological waste removal.
•Optional for Screening, but required for Quantitative analysis: Microplate reader, preferably capable of reading bichromatically at 450/620-650 nm.

Precautions

You should not use more than two strips of 8 wells at a time unless you use a multichannel pipette to add the samples, standards and other reagents, otherwise timing errors may occur.

Do not add azides to the samples or any of the reagents. Standards and some reagents contain a preservative.

Treat all reagents and samples as potentially allergenic materials.

The pH of the samples in extraction buffer ***prior*** to the extraction procedure should be in the range pH 6.8 - 7.4.

Do not pipette repeatedly from the TMB Substrate and Enzyme Conjugate bottles as this could contaminate these solutions. Determine the required volumes of these reagents and dispense the volumes required into clean test tubes just prior to use. Do not pour or return unused Enzyme Conjugate or TMB Substrate back into their bottles.
All pipette volumes should be ± 1 microlitre.
Always firmly reseal the foil bag containing the antibody-coated strips, to prevent moisture contamination.

Ensure all glassware, plasticware and storage bottles have been thoroughly cleaned to prevent any cross-contamination. If cleaning is inadequate traces of allergenic material may remain from other test kits or previous test runs. Do not use polystyrene containers as these could absorb protein from the extract. We suggest low-binding capacity plastic disposable containers.

Storage Conditions

Reagents, strips and bottled components:
Store between 2 - 8°C. ***DO NOT FREEZE ANY OF THE KIT COMPONENTS.***

Squeeze bottle containing diluted wash buffer may be stored at room temperature.
Avoid exposure of the kit and the components to direct sunlight or heat.

Reagent Preparation

Wash Solution and Extraction Solution concentrates may precipitate during refrigerated storage but will dissolve upon warming. Any precipitates must be fully dissolved prior to diluting out to the final working strength.

Wash Buffer

Remove the cap and add contents of one bottle of the 20x concentrate to distilled or deionized water to make a final volume of 500mL. Mix gently. Transfer contents of diluted wash buffer into a squeeze bottle (small tip bottle). For long term storage, label the storage bottle containing the diluted wash buffer with the kit lot number and kit expiry date.

Extraction Solution

Remove the cap and add contents of one bottle of the 20x concentrate to distilled or deionized water to make a final volume of 500mL. Mix gently. Transfer contents of diluted extraction solution into a storage bottle. For long term storage, label the storage bottle containing the diluted extraction solution with the kit lot number and kit expiry date.

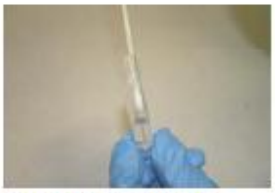
Food Allergen/Residue Swab sampling Protocol

Select a new swab tube.

Label the swab tube carefully, to identify the sample.

Place the swab tube in a suitable rack or holder.

Open a tube of swab wetting solution.



Pre-wet the swab by inserting the tip of the swab into the tube of wetting solution.

Remove excess moisture from the swab tip by pressing on the inside of the swab tube.

Swab the surface using a cross-hatch technique or according to your own protocol.

Place the swab tip into the labelled swab tube.

Cap or seal the swab tube.

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Store the sealed samples as suggested by the laboratory until ready for collection and the extraction procedure.

Sample Preparation

A representative sample(s) must be taken from the product. To ensure consistent results, a suitable blender, food processor, or a similar mixing device should be used to blend or mix the sample until it is homogenous.

Please note: A special extraction solution is required for samples containing Polyphenols, such as Dark Chocolate, Wine, Fruit Juices, Herbs, and Tannins. (Product Code: ESADDSOL).

FOR SOLID SAMPLES

A ratio of 1 part sample plus 10 parts of the prepared Extraction Solution must be used. For most samples, weigh out 5 grams of finely-ground sample into a suitable clean container for extraction purposes, and add 50mL of the working-strength Extraction Solution. If samples are considered fully homogenous and representative of a larger batch, a smaller sample volume may be used, as long as the 1 + 10 ratio is maintained. **The pH of the sample in the extraction buffer should be in the range of 6.8 - 7.4.**

Place into a water bath at 60°C for 15 minutes, and shake/mix the sample(s) for one minute every five minutes. Alternatively, if your water bath has a shake function, place the sample(s) into the water bath at 60°C for 15 minutes, and set the water bath on a gentle shake setting, to ensure the samples mix well. After the completion of the incubation and mixing stage, remove from the water bath and allow to settle and reach room temperature (20-25°C). Some samples may require centrifugation. Collect the aqueous phase from each sample - this is the sample to be tested on the kit.

FOR LIQUID SAMPLES

A ratio of 1 part sample plus 9 parts of the prepared Extraction Solution must be used for liquid samples. For most samples, place 5mL of sample into a suitable container, centrifuge tube, or similar device and add 45mL of the working-strength Extraction Solution. If samples are considered fully homogenous and representative of a larger batch, a smaller sample volume may be used, as long as the 1 + 9 ratio is maintained. **The pH of the sample in the extraction buffer should be in the range of 6.8 - 7.4.**

Place into a water bath at 60°C for 15 minutes, and shake/mix the sample(s) for one minute every five minutes. Alternatively, if your water bath has a shake function, place the sample(s) into the water bath at 60°C for 15 minutes, and set the water bath on a gentle shake setting, to ensure the samples mix well. After the completion of the incubation and mixing stage, remove from the water bath and allow to settle and reach room temperature (20-25°C). Some samples may require centrifugation. Collect the aqueous phase from each sample - this is the sample to be tested on the kit.

FOR SWAB SAMPLES

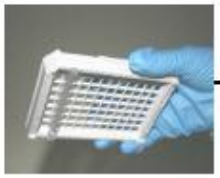
Select a new Swab tube and label carefully. Place 1mL of the diluted Extraction Solution into a clean test tube (not the Swab tube), or contact your ELISA SYSTEMS Distributor for pre-filled swab wetting tubes. Pre-moisten the Swab tip and remove excess liquid by drawing up along the inside of the tube with slight pressure. Swab the appropriate area according to your protocol. Place the Swab back into the labelled Swab tube and seal. Extract and test as soon as is possible.

To extract the material, add 1mL of the appropriate, diluted Extraction Buffer to the Swab tube and place the sealed Swab tube into a water bath at 60°C for 15 minutes, with shaking or mixing for one minute every five minutes. Vortexing is recommended. Alternatively, if your water bath has a shake function, place the sample(s) into the water bath at 60°C for 15 minutes, and set the water bath on a gentle shake setting, to ensure the samples mix well. After the completion of the incubation and mixing stage, remove from the water bath and allow to settle and cool to room temperature (20-25°C). Decant the extract into a small test tube and mix well. This is the sample to be tested on the kit.

Swab samples should be regarded as an indication of the presence/absence of the allergen protein(s) detected by this kit. Swab samples cannot be used to quantify the absolute amount of allergen proteins, but can be used as a general indication for monitoring of the levels present.

Food Allergen Residue ELISA Protocol

Add 100 microlitres of Standards and Samples to their allocated Antibody-coated wells.
Mix all wells for 10 seconds by gentle shaking on a flat surface.
Incubate for 10 minutes.

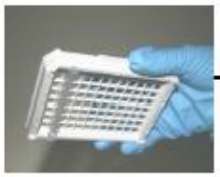


Dump liquid from wells.

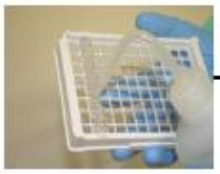
Wash wells thoroughly five times with wash buffer.

Tap wells firmly onto absorbent paper towel.

Add 100 microlitres of the Green Conjugate Solution to each well.
Mix all wells for 10 seconds by gentle shaking on a flat surface.
Incubate for 10 minutes.



Dump liquid from wells.



Wash wells thoroughly five times with wash buffer.

Tap wells firmly onto absorbent paper towel.

DO NOT WASH

Add 100 microlitres of the Substrate Solution to each well.
Mix all wells for 10 seconds by gentle shaking on a flat surface.
Incubate for 10 minutes.

Add 100 microlitres of the Stop Solution to each well.
Mix all wells for 10 seconds by gentle shaking on a flat surface.

Read results visually, comparing with the colour of the Standards.
The results can be read on a microplate/strip reader.
Results must be read within 30 minutes.

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Test Procedure - Qualitative Screening Method.

You should not use more than two strips of 8 wells at a time unless you use a multichannel pipette to add the samples, standards and other reagents, otherwise timing errors may occur.

Record the positions of the standards and samples in the assay. An example Sample Coding Sheet is supplied on Page 11 if required.
Ensure all kit components are at room temperature (20 – 25°C) prior to commencing this assay.
The negative and at least one positive standard (the 0.5 ppm standard is suggested) must be included each time the assay is run. The choice of the positive control may depend on the sample matrix being tested.

Mix the standards thoroughly prior to each use, preferably with a laboratory vortex machine.

Calculate the amount of the Enzyme Conjugate and the Substrate Solution required. Determine the number of wells to be used in the assay, multiply by 0.1mL and add about 20% of this as extra volume for pipetting purposes. Add the required amount of each of these reagents to clean, labelled tubes for use when required in the following steps. (These reagents are light-sensitive and should be protected from prolonged exposure to light).

1. Break off the number of wells needed (number of samples plus the number of standards) and place in the test strip holder. Refer to your Sample Coding Sheet for the position of the samples and the kit standards. Use a fine-tipped marker pen to place an identification mark on the tab at the end of the strip to allow for correct identification of the wells in the strip holder. Do not mark the bottoms of the wells.

2. Add 100 microlitres of the extracted test sample(s) to the correct test well(s) starting in column 1.

3. After all the samples have been added to the wells in accordance with your sample coding sheet, add 100 microlitres of the negative standard followed by 100 microlitres of the selected positive standard(s) to the appropriate wells.

   Mix wells by moving strip holder gently sideways for 10 seconds.
   Incubate at room temperature for 10 minutes, then wash.#

4 Add 100 microlitres of the Enzyme Conjugate (Green Solution) to each well.
   Mix wells by moving strip holder gently sideways for 10 seconds.
   Incubate at room temperature for 10 minutes, then wash.#

5. Add 100 microlitres of the Substrate Solution to each well.
   Mix wells by moving strip holder gently sideways for 10 seconds.
   Incubate at room temperature for 10 minutes. **DO NOT WASH AT THIS STAGE**

6. Add 100 microlitres of the Stop Solution to each well.
   Mix wells by moving strip holder gently sideways for 10 seconds.

7. Read results visually or on a microplate reader, preferably using a bichromatic reading, with the filters set at 450nm (primary) & 620-650nm (secondary/reference).
   Read Results within 30 minutes of the addition of the Stop Solution.

#Each wash cycle consists of:
1. Invert the plate and flick out the contents of each well into a sink or waste container.
2. Use the diluted wash buffer to fill each well to overflowing.
3. Invert the plate and flick out the wash solution.

Repeat steps 2 and 3 until each well has been washed five times.
Invert the wells, and tap out any residual wash solution onto absorbent paper towels.
Alternatively, use an automatic plate washer to aspirate then fill wells five times, then tap onto paper as described above.

Test Procedure - Quantitative Screening Method.

You should not use more than two strips of 8 wells at a time unless you use a multichannel pipette to add the samples, standards and other reagents, otherwise timing errors may occur.

Record the positions of the standards and samples in the assay. An example Sample Coding Sheet is supplied on Page 11 if required.
Ensure all kit components are at room temperature (20 – 25°C) prior to commencing this assay.
Standards for a Standard Curve must be included each time the assay is run.
Mix the standards thoroughly prior to each use, preferably with a laboratory vortex machine.

Calculate the amount of the Enzyme Conjugate and the Substrate Solution required. Determine the number of wells to be used in the test, multiply by 0.1mL and add about 20% of this as extra volume for pipetting purposes. Add the required amount of each of these reagents to clean, labelled tubes for use when required in the following steps. (These reagents are light-sensitive and should be protected from prolonged exposure to light).

1. Break off the number of wells needed for the samples and place in the test strip holder. Break off the number of wells for the standards and place in a separate Control Column in the strip holder. Use a fine-tipped marker pen to place an identification mark on the tab at the end of the strip to allow for correct identification of the wells in the strip holder. Do not mark the bottoms of the wells.

2. Add 100 microlitres of the extracted test sample(s) to the appropriate test well(s).

3. Add 100 microlitres of the negative standard and each of the positive standards to the appropriate test wells.

   Mix wells by moving strip holder gently sideways for 10 seconds.
   Incubate at room temperature for 10 minutes, then wash.#

4. Add 100 microlitres of the Enzyme Conjugate (Green Solution) to each well.
   Mix wells by moving strip holder gently sideways for 10 seconds.
   Incubate at room temperature for 10 minutes, then wash.#

5. Add 100 microlitres of the Substrate Solution to each well.
   Mix wells by moving strip holder gently sideways for 10 seconds.
   Incubate at room temperature for 10 minutes. DO NOT WASH AT THIS STAGE

6. Add 100 microlitres of the Stop Solution to each well.
   Mix wells by moving strip holder gently sideways for 10 seconds.

7. Read results visually or on a microplate reader, preferably using a bichromatic reading, with the filters set at 450nm (primary) & 620-650nm (secondary/reference).
   Read Results within 30 minutes of the addition of the Stop Solution.

#Each wash cycle consists of:
1. Invert the plate and flick out the contents of each well into a sink or waste container.
2. Use the diluted wash buffer to fill each well to overflowing.
3. Invert the plate and flick out the wash solution.

Repeat steps 2 and 3 until each well has been washed five times.
Invert the wells, and tap out any residual wash solution onto absorbent paper towels.
Alternatively, use an automatic plate washer to aspirate then fill wells five times, then tap onto paper as described above.

Interpretation of Results

This assay is based on comparison of colour developed in sample wells with colour developed in the supplied Control Standards. Results are expressed as ppm or mg of allergen detected per kg of sample.

NOTE: The negative standard, as well as some samples, may show some slight yellow colour. Please refer to the enclosed kit performance criteria as set out in the accompanying Certificate of Analysis for each specific lot number. The positive standard(s) should be a distinct yellow colour. If there is no yellow colour in the positive standards, the test should be regarded as invalid and should be repeated. If the positive standards again show no colour, then contact ELISA SYSTEMS immediately.

Interpretation is based on the suggested extraction/dilution protocol. **The values listed for the kit standards already take into consideration the normal extraction dilution used in this method. Therefore no additional multiplication factors of the kit standards should be used, unless the samples are extracted using a different dilution protocol to that listed in the kit method.**

For quantitation, samples should fall in the range of the standards supplied (0.5 - 5.0 ppm Hazelnut Protein). Samples may need to be diluted to achieve this result and if this occurs, remember to apply the dilution factor used in the calculation of the result.

Results are for screening purposes. **Any sample returning a positive result should be regarded as a presumptive result and confirmation or further testing should be performed.** All results should be interpreted as part of a HACCP plan for Food Allergens. Please refer to the information on Page 12.

Qualitative Method

Visual or ELISA Reader

Compare the colour or Optical Density (OD) of the sample well against the colour or OD of the chosen positive standard well. Any sample well that has a yellow colour (OD) of the same or greater intensity than the positive standard, is suspected to contain hazelnut protein at a level above the chosen cut-off value. The lowest supplied positive standard is recommended as the cut-off value for screening purposes.

Quantitative Method

ELISA Reader

Read all wells using a microstrip reader, preferably with bichromatic filters at 450nm (primary) and 620-650nm (secondary/reference).

Plot a Standard Curve using the OD values of the Control Standards (OD vs Concentration). Read the concentration of the test samples from this curve. Quantitative results are best determined in the lower to middle region of the standard curve (0.5 - 2.5 ppm).

The lower limit of Quantitation for this assay is the value of the lowest positive standard which is 0.5 ppm Hazelnut Protein. Results that indicate a value of greater than 0.25 ppm should be further investigated.

Quality Control

The use of the kit positive and negative standards allows validation of kit stability. For a valid test, the kit standards should correspond to the kit performance criteria as set out in the accompanying Certificate of Analysis for each specific lot number. Should the values fall outside these ranges as listed in the Certificate of Analysis, please contact ELISA SYSTEMS.

Trouble Shooting

Problem: Negative standard has substantial colour development.
Correction: Washings were insufficient. Repeat test with more vigorous washings.

Samples with sticky particulate matter may require more thorough washing than other samples. The potential exists for false positive results if the sample and each reagent are not thoroughly washed from the well before the addition of subsequent reagents.

Sample Coding Sheet

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Assay Name _____ Date Perfomed _____

Operator _____ Strip washing Method: [ ] Manual or [ ] Machine

Room Temperature_____ Laboratory Incubator used ? Yes/No Temperature of Incubator _____

Comments _____

_____

Assay Incubation Times: Step 1 _____ Step 2 _____ Step 3 _____ Step 4 _____

Caution: There are many combinations of formulations, additives, processes, treatments etc, that may affect the food sample and the proteins being tested. This must be considered in the application of this assay for the samples being tested and in the interpretation of the results. Choose the most appropriate positive standard for your screening. This may depend on the sample matrix being tested.

The results of the testing are only applicable to the portion of the sample product tested and to this extent, ELISA SYSTEMS cannot guarantee that hazelnut material is, or is not, present in the untested portions of the sample product.

Swab samples should be regarded as an indication of the presence/absence of the allergen protein(s) detected by this kit. Swab samples must not be used to quantify the absolute amount of allergen proteins, but should be used as a general indication for monitoring of the levels present.

Not all samples may be suitable for use with this assay. Please discuss your questions with your ELISA SYSTEMS representative.

Assays should be performed in the temperature range of 20 to 25°C.

Reference.

1. Beyer,K.,Grishina,G.,Bardina,L.,Grishin,A., Sampson,H. Identification of an 11S globulin as a major hazelnut food allergen in hazelnut induced systemic reactions. J Allergy Clin Imm. 2002;110 517-23

DISCLAIMER:

Food Allergen Kits available:

• Almond • Beta-Lactoglobulin • Buckwheat • Casein • Crustacean • Egg
• Gliadin • Hazelnut • Lupin • Mustard • Peanut • Sesame • Soy

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August 2012

1.4 Neogen Biokits Peanut

902048Q

Read instructions carefully before starting test

BioKits

Peanut Assay Kit

Store at 2–8°C (35–46°F)

Sandwich enzyme immunoassay for the detection and quantification of peanut in environmental swabs and food products by enzyme immunoassay

AOAC-RI PERFORMANCE TESTED (Certificate No. 030402)

Samples of this test kit were independently evaluated by the AOAC Research Institute and were found to perform to the producer's specifications as stated in the test kit's descriptive insert. The producer certifies this kit conforms in all respects to the specification originally evaluated by the AOAC Research Institute as detailed in Performance Tested certificate number 030402.

SPECIFICATIONS

Limit of Detection:	0.1 ppm peanut The limit of detection (LOD) was statistically determined by extrapolation of the allergen concentration at an OD value of zero (the average OD value for the zero replicates, over 10 separate assays) + 3 times the standard deviation of the OD results.
Range of Quantification:	1–20 ppm
Units of Measurement:	Peanut
Calibration:	NIST SRM2387 Peanut Butter Extract No certified reference material available
No. of Determinations:	96 (including standards and controls)
Sample Preparation:	Buffer preparation, shaking and centrifugation
Time Required:	Sample extraction time: approx. 40 minutes (5 samples) Test incubation time: 75 minutes
Specificity:	The polycolonal antibody specifically detects Conarachin (Ara h1)
Cross-reactivity:	Of a large panel of commodities including nuts, pulses, grains, proteins and seeds, none were found to be cross-reactive in the assay.

CALIBRATION

Units	Quantification
Peanut	1–20 ppm
Peanut Protein	0.25–5 ppm
Conversion Factor (% Protein)	4 (~25%*)

*Internet Symposium on Food Allergens: www.food-allergens.de

SAFETY / COSHH NOTE

"Good Laboratory Practice" techniques should be employed when using this kit; if such practices are used the reagents constitute a very low potential risk to health. Safety clothing (lab coat, glasses and gloves if necessary) should be worn and skin contact with reagents avoided; do not ingest. Any contact with skin/eyes should be treated by washing/irrigation. It is also important to be aware of the allergic, toxic or infectious potential of analytical samples.

KIT COMPONENTS

Each kit contains sufficient material for 96 measurements (including standards and controls). The following components are provided in each kit:

Component	Detail	Vials / Bottle	Ready-to-Use
Peanut Standard S1	1 ppm Peanut	1	✔
Peanut Standard S2	2 ppm Peanut	1	✔
Peanut Standard S3	5 ppm Peanut	1	✔
Peanut Standard S4	10 ppm Peanut	1	✔
Peanut Standard S5	20 ppm Peanut	1	✔
Peanut Control	USE CARE: Contains high levels of Peanut extract	1	✔
Peanut Biotin		1	✔
Avidin Peroxidase Conjugate		1	✔
TMB Substrate		1	✔
Wash Solution Concentrate		1	10-fold concentrate
Diluent Concentrate Type 8		2	5-fold concentrate
Stop Solution	USE CARE: STRONG ACID	1	✔
Peanut plate	96 Microwells (12 x 8 strips + frame)	N/A	✔
Package insert			
Blank result form			

MATERIALS REQUIRED BUT NOT PROVIDED

Reagents (all Analytical or equivalent grade)

- Sodium chloride
- Tris (hydroxymethyl) methylamine
- 1M hydrochloric acid
- Teleost gelatin (Sigma G7765)

Equipment

- Pestle and mortar, stomacher or suitable grinder/mill, WHICH CAN BE EASILY CLEANED after use to eliminate the possibility of cross-contamination between samples
- Miscellaneous laboratory plastic and/or glassware, including measuring cylinders, pipettes, disposable Pasteur pipettes, plate seals and containers suitable for food extracts
- pH Meter
- Water bath capable of maintaining 60°C ± 2°
- Wrist action or similar shaker
- Centrifuge and appropriate centrifuge/microfuge tubes for clarifying sample extract
- Precision micropipette(s) capable of delivering 50 and 100 microliters, plus disposable tips
- Microwell washer (e.g. NUNC Immuno Wash 8) or wash bottle
- Microwell plate reader, fitted with 450 nm interference filter (calibrate regularly)

PREPARATION AND EXTRACTION OF SAMPLES

Note: The assay is extremely sensitive to the presence of peanut material. As an indication, <0.5 milligrams of peanut material can be detected in approximately one kilogram of food. **However, such a limit of detection assumes that food samples can be ADEQUATELY SAMPLED prior to extraction and also that they can be rendered truly HOMOGENEOUS.**

It is strongly recommended that due note is made of the order in which samples are processed so that the likelihood of a positive result that is actually a "false positive" (e.g. when a negative sample is processed immediately after a strongly contaminated one) can be assessed.

Because of the sensitivity of the method disposable tubes/containers should be used where possible and **great care must be taken** to thoroughly clean all reusable equipment, glassware etc between samples to avoid cross contamination.

PREPARATION OF SAMPLE EXTRACTION BUFFER

Note: The same sample extracts can be used for the following assay kits: Egg, Sesame, Almond and Hazelnut.

Prepare high salt Tris extraction buffer for the extraction of food samples. Amounts quoted are for 1.5 liters, the volume required for 28 samples (~55 mL per sample to be extracted).

1. Dissolve 9.1 g (± 0.1 g) Tris and 17.5 g (± 0.1 g) sodium chloride in ~1L purified water.
2. Add 150 g gelatin (Sigma G7765) and stir well to dissolve.
3. Adjust pH to 8.15 to 8.25 with 1M hydrochloric acid; make up volume to 1,500 mL (± 10 mL).

ENZYME IMMUNOASSAY PREPARATION

1. Prepare diluted sample extracts, controls and kit materials.
2. Remove all reagents from the kit box and allow to reach ROOM TEMPERATURE (18–22°C; 64–72°F) before starting the test.
3. **Peanut standards, peanut control, peanut biotin, Avidin peroxidase conjugate, TMB substrate and stop solution** are supplied ready to use. NO PREPARATION is necessary; simply mix by repeated inversion (do not shake).

4. **Wash solution concentrate:** Supplied as a 10-fold concentrate. Dilute 1:9 in purified water to prepare working wash solution. For example add 100 mL (± 1 mL) to a volumetric flask/cylinder and make up to 1.0 L (± 10 mL) with purified water.
5. **Diluent concentrate type 8:** Supplied as a 5-fold concentrate: Dilute 1:4 in purified water to prepare working diluent solution. For 96 microwells add 60 mL (± 1 mL) to a volumetric flask/cylinder, and make up to 300 mL (± 3 mLs) with purified water. For any other number of microwells, dilute 1:4 with water, e.g. for a group of 24 microwells add 15 mL (± 0.2 mL) to 60 mL (± 1 mL) of purified water.

   Note: The diluent concentrate may produce crystals after refrigerated storage. **These crystals should be redissolved before use.** Warming to room temperature, with occasional mixing, should dissolve the salt crystals. If warming to room temperature is not sufficient, then warming to 40°C with mixing will be required.
6. **Peanut plate:** Open the foil pouch. Take out the microwell module, remove the microwell strips not required and return them to the pouch taking care that the desiccant is present. **Reseal the pouch carefully.**

   Note: With a pencil, number the columns in sequence on the upper frosted edge of the strips in use; this preserves the identity of the strips should they become detached from the frame.

PREPARATION AND EXTRACTION OF SAMPLES / CONTROLS

Sample the material to be tested; prepare by stomaching, grinding, blending or mincing. It is important to reduce the particle size of the material as far as possible, if possible to a flour or paste, while avoiding the potential for cross-contamination.

1. Weigh out at least 5 g of each sample or blank biscuit crumb control into a clean container. If appropriate (e.g. for chocolate) melt sample in water bath before adding hot extraction buffer.

Controls

- **Assay Control (recommended)**
  Spike the blank biscuit crumb with 10 microliters per gram (i.e. 50 µL for 5 g) of peanut control. Mix well and set aside for at least 30 minutes while preparing other reagents, equipment. This will prepare a spiked assay control sample containing the equivalent of ~5 ppm SRM2387 peanut butter content. Follow extraction as below.
- **Liquid Control**
  If users do not wish to prepare a spike recovery assay control using biscuit crumb, a liquid control may be prepared by diluting 10 µL of peanut control in 10 mL of extraction buffer. This dilution must be diluted further (1:9 in working diluent) prior to assay, e.g., add 100 µL (± 1 µL) of diluted control to 900 µL (± 9 µL) of working diluent in a clean glass or plastic container. Mix/vortex well.

2. For most samples, pre-heat extraction buffer to 60°C in a water bath. For ice cream samples, use extraction buffer at room temperature.

   Note: If hot buffer is added accidentally to ice cream only very minor reductions in peanut content will be observed, although use of only cold buffer was tested in the AOAC-RI trial.
3. Add hot or cold extraction buffer at a ratio of 10 mL (± 0.1 mL) per gram of sample into the container containing sample to be extracted.
4. Shake for 15 minutes at room temperature.
5. Remove a portion of the extracted sample slurry with a disposable Pasteur pipette, place into an Eppendorf tube, seal and centrifuge at ~10,000 g for 10 minutes. Alternatively, allow the mixture to settle until a liquid extract layer appears.

6. With a clean disposable pipette, remove a portion of the aqueous extract from below any fat that might be present and above the settled layer; place into a second, clean tube/container. Mix/vortex well.
7. Dilute the settled/centrifuged sample or control extracts 1:9 in working diluent solution, e.g. add 100 µL (±1 µL) of extract to 900 µL (± 9 µL) of working diluent in a clean glass/plastic container. Mix/vortex well.
8. The diluted sample extract is now ready for testing.

ENVIRONMENTAL SWAB SAMPLE PREPARATION

For the preparation of environmental swab samples, the BioKits Allergen Swabbing Kit (BASK) is required (Item No. 901042J). This kit can be used in conjunction with the Peanut Assay Kit for the determination of peanut contamination levels in the environment.

TEST PROCEDURE

It is recommended that, for practice assays, small runs are performed and duplicate wells used for all samples. When good precision is being achieved (replicate OD450 nm %CVs < ~15%) reaction wells may be run singly. However, it is good laboratory practice that duplicates are run for some or all diluted extracts and imprecision (%CV of OD450 nm and concentration values) estimated in all assays as part of an ongoing QC program.

Note: When testing has been started, all steps should be completed without interruption to reduce the possibility of assay "drift" across the microwells.

1. Prepare diluted sample extracts and kit materials.
2. Add 100 µL (±1 µL) of working diluent (used as zero standard), peanut standard(s) S1–S5 and each of the diluted assay controls and sample extracts into appropriate microwells.

   Note: Use a separate disposable tip for pipetting each Standard/diluted Sample Extract to prevent cross-contamination.
3. Incubate for 30 MINUTES at room temperature, static (without shaking).
4. Discard the liquid from the microwells, then (using NUNC Immuno Wash 8 or wash bottle) completely fill all wells with working wash solution. Discard the liquid and repeat the fill and discard sequence 4 more times (5 washes in total). Following the final discard, tap the plate upside down on several layers of absorbent tissue to completely remove residual droplets/bubbles of wash solution.
5. Add 50 µL (± 0.5 µL) of peanut biotin to all wells.
6. Mix the strips in the frame gently by hand on a flat surface and allow to incubate for 15 MINUTES at room temperature, static.
7. At the end of the incubation, repeat the wash sequence used in Step 4.
8. Add 50 µL (± 0.5 µL) of AVIDIN PEROXIDASE CONJUGATE to all wells. Work from top to bottom of each strip as previously described.
9. Mix the strips in the frame gently by hand on a flat surface and allow to incubate for 15 MINUTES at room temperature.
10. At the end of the incubation, repeat the wash sequence described in Step 4.
11. Add 100 µL (± 1 µL) of TMB substrate to all wells.
12. Mix the strips gently by hand on a flat surface and incubate for 15 MINUTES at room temperature, static.

    Note: The rate of color development is dependent on laboratory conditions and should be monitored in order to obtain suitable OD450 nm levels.

13. Add 50 µL (± 0.5 µL) of stop solution to all wells. Mix gently by hand to distribute the stop solution and prevent further color development. Color changes from blue to yellow and intensifies.
14. Using a microplate reader fitted with a 450 nm filter blank the reader on "Air" then measure and record the absorbance of each of the microwells.

    Note: Readings should be completed within 10 minutes of adding stop solution.

RESULTS

Qualitative

For qualitative assessment an individual peanut standard can be used to define a specific (X ppm) cutoff level from the average OD450 nm.

Samples with absorbance values below the cutoff are classified as: NEGATIVE <X ppm

Samples at or above the cutoff are classified as: POSITIVE >X ppm

Quantitative

Quantitative estimates of peanut content can be obtained by using a calibration curve; to construct the calibration curve, use two-cycle log graph paper. Plot the mean absorbance value for each of the peanut standards (1, 2, 5, 10 and 20 ppm) and fit a best curve to join each neighboring point. Alternatively, the results can be calculated using a graphical data reduction package using a suitable line fit for the curve. A linear regression (y=mX + C) can be used if only standards S1 to S4 are plotted.

Values returned when interpolating off the peanut assay curve line correspond approximately to peanut content in the ORIGINAL SAMPLE (assuming that the nominal extraction and dilution conditions are adhered to). If additional dilutions are performed then the necessary factor needs to be applied to the recorded peanut content.

Report samples with an OD450 < Standard S1 as "<LOQ".

Report samples with an OD450 > Standard S5 as ">20 ppm".

Poor replication (e.g. six zero standard wells %CV >12.5–15%) may indicate inadequate washing, contamination of the TMB substrate or splashing of Avidin peroxidase. Such imprecision is an indication of a problem during the performance of the assay, which may be INVALID and need to be repeated.

INTERPRETATION

The variability of raw material/product sampling, food composition (salinity, acidity, etc.) treatment of foodstuffs during processing (heat, pressure, etc.), difficulty of obtaining complete homogeneity during extraction and the reactivity of different sources of peanut material means that the amount of detectable peanut protein in the extract may vary considerably.

Note: If a food sample gives a negative result in the test **it may still contain peanut material** which is either unreactive in the test or below the limit of detection. **It should not be assumed that the food is "peanut-free".**

ESTIMATION OF PEANUT PROTEIN CONTENT FROM SWAB SAMPLES

Approximate estimates of the quantity of peanut present in the swab solution are taken from the peanut calibration curve as follows:

A swab sample (no dilution) giving an absorbance which extrapolates to 2 ppm on the peanut standard curve contains 20 ng/mL peanut. This factor of x10 and conversion to ng/mL can be applied to any undiluted swab sample which gives an absorbance value which falls within the quantifiable range (1–20 ppm).

Because of the variability of the swabbing process, the amount of detectable protein in the swabbing solution may vary considerably. Recoveries of various allergens from a swabbed area vary quite widely and detection of allergens/peanut from complex and/or highly processed food sources can be difficult. For further information see the Biokits Allergen Swabbing Kit insert.

SHELF LIFE

Diluted wash buffer: Once diluted 1:9 the wash buffer is stable at room temperature in a sealed clean container for at least 1 week.

Diluted assay diluent: Fresh assay diluent should be prepared for each assay.

Extraction buffer: Fresh extraction buffer should be prepared daily.

Extracted samples: The undiluted sample extracts may be stored at 2–8ºC (35–46°C) for up to five days. If prolonged storage is required the undiluted extracts must be kept frozen (< 20ºC) where they are stable for several months.

Kit reagents: The kit should be stored at 2–8ºC (35–46°C). The shelf life of unopened kit components is indicated by the expiry date on the respective labels. Once the kit reagents have been opened, exposure to elevated (i.e. room) temperatures should be minimized.

Peanut plate: must be kept dry. Keep sealed in foil pouch with desiccant.

Providing these instructions are complied with the opened kit reagents should be stable for many weeks or months at 2–8ºC (35–46°C).

PERFORMANCE CHARACTERISTICS

The assay is designed to give optimum performance at ambient temperature (18–22ºC; 64–72°F).

- Standard S1 OD450 nm should be greater than 1.75 x zero OD450 nm.
- Standard S4 OD450 nm preferably >1.2 absorbance units.
- Peanut control should read off the standard curve within the specifications printed on the certificate of analysis that accompanies this kit.
- At temperatures below 18ºC or above 22ºC incubations may need to be lengthened or reduced respectively to maintain performance).

Poor replication (e.g. six zero standard wells %CV >12.5–15%) may indicate inadequate washing, contamination of the TMB substrate or splashing of Avidin peroxidase. Such imprecision is an indication of a problem during the performance of the assay, which may be INVALID and need to be repeated.

Peanut Assay Kit AOAC-RI performance tested validation report available on request

CUSTOMER SERVICE

Neogen Customer Assistance and Technical Service can be reached between 8 a.m. and 6 p.m. Eastern time by calling 800/234-5333 or 517/372-9200 and asking for a Neogen sales representative or Technical Services. Assistance is available on a 24-hour basis by calling 800/234-5333. Training on this product, and all Neogen test kits, is available.

MSDS INFORMATION AVAILABLE

Material safety data sheets (MSDS) are available for this test kit, and all of Neogen's Food Safety test kits, at www.neogen.com, or by calling Neogen at 800/234-5333 or 517/372-9200.

WARRANTY

Neogen Corporation makes no warranty of any kind, either expressed or implied, except that the materials from which its products are made are of standard quality. If any materials are defective, Neogen will provide a replacement product. Buyer assumes all risk and liability resulting from the use of this product. There is no warranty of merchantability of this product, or of the fitness of the product for any purpose. Neogen shall not be liable for any damages, including special or consequential damage, or expense arising directly or indirectly from the use of this product.

TESTING KITS AVAILABLE FROM NEOGEN

Natural Toxins
- Aflatoxin, DON, Ochratoxin, Zearalenone, T-2/HT-2 Toxins, Fumonisin, Histamine

Foodborne Bacteria
- *E. coli* 0157:H7, *Salmonella*, *Listeria*, *Listeria monocytogenes*, *Campylobacter*, *Staphylococcus aureus*

Sanitation
- ATP, Yeast and Mold, Total Plate Count, Generic *E. coli* and Total Coliforms, Protein Residues

Food Allergens
- Peanut, Milk, Egg, Almond, Gliadin, Soy, Hazelnut, Mustard, Sesame, Shellfish, Walnut

Genetic Modification
- CP4 (Roundup Ready®)

Ruminant By-products
- Meat and Bone Meal, Feed

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T201314 BioKitsPeanutAssay_0510

1.5 **ELISA Systems Peanut Residue**

Peanut Residue

Product Code: ESPRDT-48

Microwell ELISA For Laboratory Use Only. Store Between 2 - 8°C

For screening for the presence of peanut protein residue in food products and environmental samples.

Directions For Use

Intended Use

The ELISA SYSTEMS Peanut Residue assay is an enzyme-linked immunosorbent assay (ELISA) that may be used to screen food products for the presence of peanut material. This assay is a rapid and reliable test that significantly reduces the time required to screen food products for the presence of peanut residues.

Background

Peanuts are a major cause of food allergies both in children and in adults and can induce anaphylactic shock[1]. In recent years, peanut allergens have been identified and characterized. The proteins *Ara h1* and *Ara h2* have been the focus of much attention[2].

Because of their allergenic properties, *Ara h1* and *Ara h2* were chosen as the target proteins for the ELISA SYSTEMS Peanut Residue ELISA.

Please note: A special extraction solution is required for samples containing Polyphenols, including Dark Chocolate, Wine, Fruit Juices, Herbs, and Tannins. (Product Code: ESADDSOL).

Principle of Procedure

The ELISA SYSTEMS Peanut Residue ELISA is a double antibody (sandwich) ELISA utilizing anti-peanut protein antibodies coated onto microwells. After addition of the sample, the Enzyme Conjugate, then the TMB Substrate, a positive reaction (indicating the presence of peanut protein) produces a blue colour. Addition of the Stop Solution ends the assay and turns the blue colour to yellow. The results may be read visually or with an ELISA reader.

Comparison of the samples with the supplied positive standards allows estimation of the levels o total peanut protein present in the sample, based on an average composition of peanut proteins. The variation of protein levels in different strains must be taken into consideration when assessing the potential total peanut protein concentration and the allergenic issues associated with the sample being tested.

The results of the testing are only applicable to the portion of the sample product tested and to this extent, ELISA SYSTEMS cannot guarantee that peanut material is, or is not, present in the untested portions of the sample product.

This assay is designed for screening purposes.
Any sample returning a positive result should be regarded as a presumptive result and confirmation or further testing should be performed.

Please note: A special extraction solution is required for samples containing Polyphenols, such as Dark Chocolate, Wine, Fruit Juices, Herbs, and Tannins. (Product code: ESADDSOL). One bottle of 20x Concentrate is supplied with this kit.

How the ELISA SYSTEMS Peanut Residue test works:

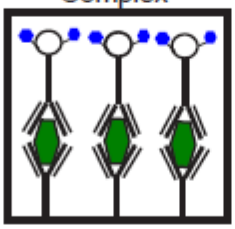
Step 1

Sample is added

The test sample is added and if peanut residue is present, it will bind to the specific antibodies.

Step 2

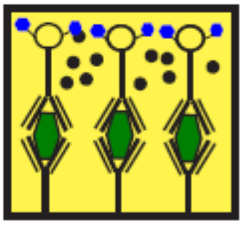
Antigen-Antibody Complex



Enzyme-labelled Conjugate is added and binds to the captured peanut residue to form a “sandwich”.

Step 3

Coloured End-Point



TMB Substrate is added, which is converted in the presence of the EnzymeConjugate to form a blue colour if peanut residue is present in the sample.

A yellow colour is formed once Acid is added to stop the reaction.

Reagents Supplied

Test Strips: microwells coated with anti-Peanut Protein antibodies – 48 wells.
Test strip holder: One (1)

Negative Control Standard: One (1) vial containing 1.7mL of a buffered base.

Positive Control Standards:
One (1) vial containing 1.7mL of Peanut Protein in a buffer to provide a Control value of 1.0 ppm
One (1) vial containing 1.7mL of Peanut Protein in a buffer to provide a Control value of 2.5 ppm
One (1) vial containing 1.7mL of Peanut Protein in a buffer to provide a Control value of 5.0 ppm
One (1) vial containing 1.7mL of Peanut Protein in a buffer to provide a Control value of 10.0 ppm
One (1) vial containing 1.7mL of Peanut Protein in a buffer to provide a Control value of 15.0 ppm

Enzyme Conjugate: One (1) bottle containing 7mL of Peroxidase-conjugated anti-Peanut Protein polyclonal antibodies with preservative.
Please note - this reagent is light-sensitive. Avoid unnecessary exposure of the reagent to light.

Substrate: One (1) bottle containing 7mL of a stabilized Tetramethylbenzidine (TMB).
Please note - this reagent is light-sensitive. Avoid unnecessary exposure of the reagent to light.

Wash Buffer Solution concentrate (20x): Three (3) bottles containing 25mL each of concentrated wash buffer solution with preservative.

Extraction Solution concentrate (20x): Three (3) bottles containing 25mL each of concentrated extraction solution with preservative.

Extraction Solution concentrate (20x) for samples containing Polyphenols:
(ESADDSOL). One (1) bottle containing 20x Enhanced Extraction Solution concentrate.

Stop Solution: One (1) bottle containing 7mL of 1M Phosphoric acid.
(CAUTION THIS SOLUTION IS ACIDIC) Avoid contact of this solution with eyes and skin. In case of skin contact, wash immediately with copious amounts of water. A mild soap should be used. In case of eye contact, flush generously for at least 15 minutes with water. Seek urgent medical attention if the irritation persists or is severe.

Additional Materials Required:

•Suitable clean containers for use in the sample extraction procedure. Do not use polystyrene containers as these could absorb protein from the extract. We suggest low-binding capacity plastic disposable containers.
•Clean test tubes or small microtubes for aliquotting the Enzyme Conjugate and Substrate volumes prior to use. •Data record sheets. •Fine-tipped marking-pen. •Laboratory Timer. •Paper towels.
•Plastic wash bottle with a fine tip. •Distilled or Deionized water. •Laboratory Vortex machine. •Laboratory balance capable of measuring at least 2 decimal places.
•Pipettes: 20-200 microlitre; 100-1000 microlitre (optional, for aliquoting reagents); 20-200 microlitre multichannel pipette if using more than two strips per run; disposable tips.
•Water Bath, capable of heating and holding the samples at 60°C during extraction. •Blender, Grinder, Stomacher, Ultraturrax or similar devices for sample preparation. •Disinfecting Solution or a system for Biological waste removal.
•Optional for Screening, but required for Quantitative analysis: Microplate reader, preferably capable of reading bichromatically at 450/620-650 nm.

Precautions

You should not use more than two strips of 8 wells at a time unless you use a multichannel pipette to add the samples, standards and other reagents, otherwise timing errors may occur.

Do not add azides to the samples or any of the reagents. Standards and some reagents contain a preservative.

Treat all reagents and samples as potentially allergenic materials.

The pH of the samples in extraction buffer ***prior*** to the extraction procedure should be in the range pH 6.8 - 7.4.

Do not pipette repeatedly from the TMB Substrate and Enzyme Conjugate bottles as this could contaminate these solutions. Determine the required volumes of these reagents and dispense the volumes required into clean test tubes just prior to use. Do not pour or return unused Enzyme Conjugate or TMB Substrate back into their bottles.
All pipette volumes should be ± 1 microlitre.

Always firmly reseal the foil bag containing the antibody-coated strips, to prevent moisture contamination.

Ensure all glassware, plasticware and storage bottles have been thoroughly cleaned to prevent any cross-contamination. If cleaning is inadequate traces of allergenic material may remain from other test kits or previous test runs. Do not use polystyrene containers as these could absorb protein from the extract. We suggest low-binding capacity plastic disposable containers.

Storage Conditions

Reagents, strips and bottled components:
Store between 2 - 8°C. ***DO NOT FREEZE ANY OF THE KIT COMPONENTS.***

Squeeze bottle containing diluted wash buffer may be stored at room temperature.
Avoid exposure of the kit and the components to direct sunlight or heat.

Reagent Preparation

Wash Solution and Extraction Solution concentrates may precipitate during refrigerated storage but will dissolve upon warming. Any precipitates must be fully dissolved prior to diluting out to the final working strength.

Wash Buffer

Remove the cap and add contents of one bottle of the 20x concentrate to distilled or deionized water to make a final volume of 500mL. Mix gently. Transfer contents of diluted wash buffer into a squeeze bottle (small tip bottle). For long term storage, label the storage bottle containing the diluted wash buffer with the kit lot number and kit expiry date.

Extraction Solution

Remove the cap and add contents of one bottle of the 20x concentrate to distilled or deionized water to make a final volume of 500mL. Mix gently. Transfer contents of diluted extraction solution into a storage bottle. For long term storage, label the storage bottle containing the diluted extraction solution with the kit lot number and kit expiry date.

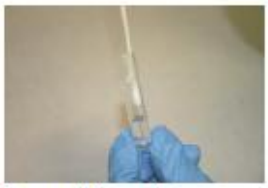
Food Allergen/Residue Swab sampling Protocol

Select a new swab tube.

Label the swab tube carefully, to identify the sample.

Place the swab tube in a suitable rack or holder.

Open a tube of swab wetting solution.



Pre-wet the swab by inserting the tip of the swab into the tube of wetting solution.

Remove excess moisture from the swab tip by pressing on the inside of the swab tube.

Swab the surface using a cross-hatch technique or according to your own protocol.

Place the swab tip into the labelled swab tube.

Cap or seal the swab tube.

ELISA Systems

Store the sealed samples as suggested by the laboratory until ready for collection and the extraction procedure.

Sample Preparation

A representative sample(s) must be taken from the product. To ensure consistent results, a suitable blender, food processor, or a similar mixing device should be used to blend or mix the sample until it is homogenous.

Please note: A special extraction solution is required for samples containing Polyphenols, such as Dark Chocolate, Wine, Fruit Juices, Herbs, and Tannins. (Product Code: ESADDSOL).

FOR SOLID SAMPLES

A ratio of 1 part sample plus 10 parts of the prepared Extraction Solution must be used. For most samples, weigh out 5 grams of finely-ground sample into a suitable clean container for extraction purposes, and add 50mL of the working-strength Extraction Solution. If samples are considered fully homogenous and representative of a larger batch, a smaller sample volume may be used, as long as the 1 + 10 ratio is maintained. **The pH of the sample in the extraction buffer should be in the range of 6.8 - 7.4.**

Place into a water bath at 60°C for 15 minutes, and shake/mix the sample(s) for one minute every five minutes. Alternatively, if your water bath has a shake function, place the sample(s) into the water bath at 60°C for 15 minutes, and set the water bath on a gentle shake setting, to ensure the samples mix well. After the completion of the incubation and mixing stage, remove from the water bath and allow to settle and reach room temperature (20-25°C). Some samples may require centrifugation. Collect the aqueous phase from each sample - this is the sample to be tested on the kit.

FOR LIQUID SAMPLES

A ratio of 1 part sample plus 9 parts of the prepared Extraction Solution must be used for liquid samples. For most samples, place 5mL of sample into a suitable container, centrifuge tube, or similar device and add 45mL of the working-strength Extraction Solution. If samples are considered fully homogenous and representative of a larger batch, a smaller sample volume may be used, as long as the 1 + 9 ratio is maintained. **The pH of the sample in the extraction buffer should be in the range of 6.8 - 7.4.**

Place into a water bath at 60°C for 15 minutes, and shake/mix the sample(s) for one minute every five minutes. Alternatively, if your water bath has a shake function, place the sample(s) into the water bath at 60°C for 15 minutes, and set the water bath on a gentle shake setting, to ensure the samples mix well. After the completion of the incubation and mixing stage, remove from the water bath and allow to settle and reach room temperature (20-25°C). Some samples may require centrifugation. Collect the aqueous phase from each sample - this is the sample to be tested on the kit.

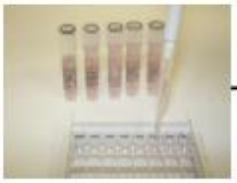
FOR SWAB SAMPLES

Select a new Swab tube and label carefully. Place 1mL of the diluted Extraction Solution into a clean test tube (not the Swab tube), or contact your ELISA SYSTEMS Distributor for pre-filled swab wetting tubes. Pre-moisten the Swab tip and remove excess liquid by drawing up along the inside of the tube with slight pressure. Swab the appropriate area according to your protocol. Place the Swab back into the labelled Swab tube and seal. Extract and test as soon as is possible.

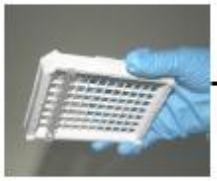
To extract the material, add 1mL of the appropriate, diluted Extraction Buffer to the Swab tube and place the sealed Swab tube into a water bath at 60°C for 15 minutes, with shaking or mixing for one minute every five minutes. Vortexing is recommended. Alternatively, if your water bath has a shake function, place the sample(s) into the water bath at 60°C for 15 minutes, and set the water bath on a gentle shake setting, to ensure the samples mix well. After the completion of the incubation and mixing stage, remove from the water bath and allow to settle and cool to room temperature (20-25°C). Decant the extract into a small test tube and mix well. This is the sample to be tested on the kit.

Swab samples should be regarded as an indication of the presence/absence of the allergen protein(s) detected by this kit. Swab samples cannot be used to quantify the absolute amount of allergen proteins, but can be used as a general indication for monitoring of the levels present.

Food Allergen Residue ELISA Protocol



Add 100 microlitres of Standards and Samples to their allocated Antibody-coated wells.
Mix all wells for 10 seconds by gentle shaking on a flat surface.
Incubate for 10 minutes.

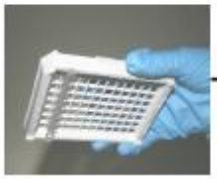


Dump liquid from wells.

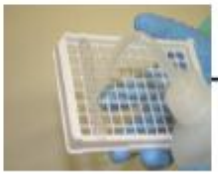
Wash wells thoroughly five times with wash buffer.

Tap wells firmly onto absorbent paper towel.

Add 100 microlitres of the Green Conjugate Solution to each well.
Mix all wells for 10 seconds by gentle shaking on a flat surface.
Incubate for 10 minutes.



Dump liquid from wells.



Wash wells thoroughly five times with wash buffer.

Tap wells firmly onto absorbent paper towel.

DO NOT WASH

Add 100 microlitres of the Substrate Solution to each well.
Mix all wells for 10 seconds by gentle shaking on a flat surface.
Incubate for 10 minutes.

Add 100 microlitres of the Stop Solution to each well.
Mix all wells for 10 seconds by gentle shaking on a flat surface.

Read results visually, comparing with the colour of the Standards.
The results can be read on a microplate/strip reader.
Results must be read within 30 minutes.

ELISA Systems

Test Procedure - Qualitative Screening Method.

You should not use more than two strips of 8 wells at a time unless you use a multichannel pipette to add the samples, standards and other reagents, otherwise timing errors may occur.

Record the positions of the standards and samples in the assay. An example Sample Coding Sheet is supplied on Page 11 if required.
Ensure all kit components are at room temperature (20 – 25°C) prior to commencing this assay.
The negative and at least one positive standard (the 1.0 ppm standard is suggested) must be included each time the assay is run. The choice of the positive control may depend on the sample matrix being tested.

Mix the standards thoroughly prior to each use, preferably with a laboratory vortex machine.

Calculate the amount of the Enzyme Conjugate and the Substrate Solution required. Determine the number of wells to be used in the assay, multiply by 0.1mL and add about 20% of this as extra volume for pipetting purposes. Add the required amount of each of these reagents to clean, labelled tubes for use when required in the following steps. (These reagents are light-sensitive and should be protected from prolonged exposure to light).

1. Break off the number of wells needed (number of samples plus the number of standards) and place in the test strip holder. Refer to your Sample Coding Sheet for the position of the samples and the kit standards. Use a fine-tipped marker pen to place an identification mark on the tab at the end of the strip to allow for correct identification of the wells in the strip holder. Do not mark the bottoms of the wells.

2. Add 100 microlitres of the extracted test sample(s) to the correct test well(s) starting in column 1.

3. After all the samples have been added to the wells in accordance with your sample coding sheet, add 100 microlitres of the negative standard followed by 100 microlitres of the selected positive standard(s) to the appropriate wells.

   Mix wells by moving strip holder gently sideways for 10 seconds.
   Incubate at room temperature for 10 minutes, then wash.#

4 Add 100 microlitres of the Enzyme Conjugate (Green Solution) to each well.
   Mix wells by moving strip holder gently sideways for 10 seconds.
   Incubate at room temperature for 10 minutes, then wash.#

5. Add 100 microlitres of the Substrate Solution to each well.
   Mix wells by moving strip holder gently sideways for 10 seconds.
   Incubate at room temperature for 10 minutes. DO NOT WASH AT THIS STAGE

6. Add 100 microlitres of the Stop Solution to each well.
   Mix wells by moving strip holder gently sideways for 10 seconds.

7. Read results visually or on a microplate reader, preferably using a bichromatic reading, with the filters set at 450nm (primary) & 620-650nm (secondary/reference).
   Read Results within 30 minutes of the addition of the Stop Solution.

#Each wash cycle consists of:
1. Invert the plate and flick out the contents of each well into a sink or waste container.
2. Use the diluted wash buffer to fill each well to overflowing.
3. Invert the plate and flick out the wash solution.
Repeat steps 2 and 3 until each well has been washed five times.
Invert the wells, and tap out any residual wash solution onto absorbent paper towels.
Alternatively, use an automatic plate washer to aspirate then fill wells five times, then tap onto paper as described above.

Test Procedure - Quantitative Screening Method.

You should not use more than two strips of 8 wells at a time unless you use a multichannel pipette to add the samples, standards and other reagents, otherwise timing errors may occur.

Record the positions of the standards and samples in the assay. An example Sample Coding Sheet is supplied on Page 11 if required.
Ensure all kit components are at room temperature (20 – 25°C) prior to commencing this assay.
Standards for a Standard Curve must be included each time the assay is run.
Mix the standards thoroughly prior to each use, preferably with a laboratory vortex machine.

Calculate the amount of the Enzyme Conjugate and the Substrate Solution required. Determine the number of wells to be used in the test, multiply by 0.1mL and add about 20% of this as extra volume for pipetting purposes. Add the required amount of each of these reagents to clean, labelled tubes for use when required in the following steps. (These reagents are light-sensitive and should be protected from prolonged exposure to light).

1. Break off the number of wells needed for the samples and place in the test strip holder. Break off the number of wells for the standards and place in a separate Control Column in the strip holder. Use a fine-tipped marker pen to place an identification mark on the tab at the end of the strip to allow for correct identification of the wells in the strip holder. Do not mark the bottoms of the wells.

2. Add 100 microlitres of the extracted test sample(s) to the appropriate test well(s).

3. Add 100 microlitres of the negative standard and each of the positive standards to the appropriate test wells.

   Mix wells by moving strip holder gently sideways for 10 seconds.
   Incubate at room temperature for 10 minutes, then wash.#

4. Add 100 microlitres of the Enzyme Conjugate (Green Solution) to each well.
   Mix wells by moving strip holder gently sideways for 10 seconds.
   Incubate at room temperature for 10 minutes, then wash.#

5. Add 100 microlitres of the Substrate Solution to each well.
   Mix wells by moving strip holder gently sideways for 10 seconds.
   Incubate at room temperature for 10 minutes. DO NOT WASH AT THIS STAGE

6. Add 100 microlitres of the Stop Solution to each well.
   Mix wells by moving strip holder gently sideways for 10 seconds.

7. Read results visually or on a microplate reader, preferably using a bichromatic reading, with the filters set at 450nm (primary) & 620-650nm (secondary/reference).
   Read Results within 30 minutes of the addition of the Stop Solution.

#Each wash cycle consists of:
1. Invert the plate and flick out the contents of each well into a sink or waste container.
2. Use the diluted wash buffer to fill each well to overflowing.
3. Invert the plate and flick out the wash solution.

Repeat steps 2 and 3 until each well has been washed five times.
Invert the wells, and tap out any residual wash solution onto absorbent paper towels.
Alternatively, use an automatic plate washer to aspirate then fill wells five times, then tap onto paper as described above.

Interpretation of Results

This assay is based on comparison of colour developed in sample wells with colour developed in the supplied Control Standards. Results are expressed as ppm or mg of allergen detected per kg of sample.

NOTE: The negative standard, as well as some samples, may show some slight yellow colour. Please refer to the enclosed kit performance criteria as set out in the accompanying Certificate of Analysis for each specific lot number. The positive standard(s) should be a distinct yellow colour. If there is no yellow colour in the positive standards, the test should be regarded as invalid and should be repeated. If the positive standards again show no colour, then contact ELISA SYSTEMS immediately.

Interpretation is based on the suggested extraction/dilution protocol. **The values listed for the kit standards already take into consideration the normal extraction dilution used in this method. Therefore no additional multiplication factors of the kit standards should be used, unless the samples are extracted using a different dilution protocol to that listed in the kit method.**

For quantitation, samples should fall in the range of the standards supplied (1.0 - 15.0 ppm Peanut Protein). Samples may need to be diluted to achieve this result and if this occurs, remember to apply the dilution factor used in the calculation of the result.

Results are for screening purposes. **Any sample returning a positive result should be regarded as a presumptive result and confirmation or further testing should be performed.** All results should be interpreted as part of a HACCP plan for Food Allergens. Please refer to the information on Page 12.

Qualitative Method

Visual or ELISA Reader

Compare the colour or Optical Density (OD) of the sample well against the colour or OD of the chosen positive standard well. Any sample well that has a yellow colour (OD) of the same or greater intensity than the positive standard, is suspected to contain peanut protein at a level above the chosen cut-off value. The lowest supplied positive standard is recommended as the cut-off value for screening purposes.

Quantitative Method

ELISA Reader

Read all wells using a microstrip reader, preferably with bichromatic filters at 450nm (primary) and 620-650nm (secondary/reference).

Plot a Standard Curve using the OD values of the Control Standards (OD vs Concentration). Read the concentration of the test samples from this curve. Quantitative results are best determined in the lower to middle region of the standard curve (1.0 - 10.0 ppm).

The lower limit of Quantitation for this assay is the value of the lowest positive standard which is 1.0 ppm peanut protein. Results that indicate a value of greater than 0.50 ppm should be further investigated.

Quality Control

The use of the kit positive and negative standards allows validation of kit stability. For a valid test, the kit standards should correspond to the kit performance criteria as set out in the accompanying Certificate of Analysis for each specific lot number. Should the values fall outside these ranges as listed in the Certificate of Analysis, please contact ELISA SYSTEMS.

Trouble Shooting

Problem: Negative standard has substantial colour development.
Correction: Washings were insufficient. Repeat test with more vigorous washings.

Samples with sticky particulate matter may require more thorough washing than other samples. The potential exists for false positive results if the sample and each reagent are not thoroughly washed from the well before the addition of subsequent reagents.

Sample Coding Sheet

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Assay Name _____ Date Perfomed _____

Operator _____ Strip washing Method: [ ] Manual or [ ] Machine

Room Temperature_____ Laboratory Incubator used ? Yes/No Temperature of Incubator _____

Comments _____

Assay Incubation Times: Step 1 _____ Step 2 _____ Step 3 _____ Step 4 _____

Caution: There are many combinations of formulations, additives, processes, treatments, etc, that may affect the food sample and the proteins being tested. This must be considered in the application of this assay for the samples being tested and in the interpretation of the results. Choose the most appropriate positive standard for your screening. This may depend on the sample matrix being tested.

The results of the testing are only applicable to the portion of the sample product tested and to this extent, ELISA SYSTEMS cannot guarantee that peanut material is, or is not, present in the untested portions of the sample product.

Swab samples should be regarded as an indication of the presence/absence of the allergen protein(s) detected by this kit. Swab samples must not be used to quantify the absolute amount of allergen proteins, but should be used as a general indication for monitoring of the levels present.

Not all samples may be suitable for use with this assay. Please discuss your questions with your ELISA SYSTEMS representative.

Assays should be performed in the temperature range of 20 to 25°C.

References

1. De Jong,E.C., Van Zijverden,M., Spanhaak, S., Koppelman, S.J., Pellegrom, H., and Penninks, A.H. (1998) Identification and partial characterization of multiple major allergens in peanut proteins. Clin.Exp. Allergy 28, 743-751.
2. Koppelman,S.J., Vlooswijk,R.A., Knippels,L.M.,Hessing,M., Knol,E.F., van Reijsen,F.C. and Bruijnzeel-Komen, C.A. (2001) Quantification of major peanut allergens Ara h 1 and Ara h 2 in the peanut varieties Runner, Spanish, Virginia and Valencia, bred in different parts of the world. Allergy, 56, 132-137.

DISCLAIMER:

ELISA SYSTEMS excludes all representations, warranties, conditions and promises of any kind (express or implied) in relation to the product supplied ("the Product"), including any warranty or conditions in relation to the quality, fitness or suitability of the Product, except for any warranties which, by law, ELISA SYSTEMS cannot exclude. The Buyer assumes all risk and liability for the Product, its use or the fitness of the Product for any purpose.

In any event, ELISA SYSTEMS' liability for breaching any implied warranty or conditions is limited to the replacement of the Product.

Food Allergen Kits available:

• Almond • Beta-Lactoglobulin • Buckwheat • Casein • Crustacean • Egg
• Gliadin • Hazelnut • Lupin • Mustard • Peanut • Sesame • Soy

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www.elisasystems.net

January 2014

2 FSA Letters to interested parties and retailers

2.1 Letter to Retailers

THE FOOD STANDARDS AGENCY IS CARRYING OUT A SURVEY OF ALLERGEN ADVISORY LABELLING AND ALLERGEN CONTENT OF UK RETAIL FOODS

The Food Standards Agency (FSA) is currently undertaking a survey to investigate allergen advisory labelling and allergen content of pre-packed processed foods sold in the UK.

This leaflet has been given to you by a member of Ventress Technical Ltd who is collecting samples on behalf of the FSA. They have obtained samples of a product/(s) from your retail establishment as part of this survey.

This leaflet explains why the FSA undertakes food surveys and how it will publish the results.

Why do the FSA carry out food surveys?

The FSA conducts regular surveys to:

- check that food is safe for consumers and that they are not being misled
- collect information on the composition of food
- help develop the FSA's polices on food safety
- check if current laws, codes of practice and other measures are working.

What products will be sampled and tested for the current survey?

A broad range of pre-packed[1] processed foods with and without allergen advisory labelling will be sampled and tested for the presence and level of one or more of the following four food allergens: milk, gluten, peanut and hazelnut.

Why has the FSA chosen to purchase samples from your establishment?

Your establishment has been selected at random because it is one of the places where people buy the type of samples the FSA are looking at in their current survey of allergen advisory labelling.

How will I know which products have been taken?

A list of all the samples taken for the survey will be held by the FSA. Details of the samples purchased from an establishment will be sent to the brand owner[2] as soon as practicable.

[1] Pre-packed foods are foods that have been put into packaging before sale. They are usually produced at a site separate from where the product is sold to the customer. Most foods sold in retail stores will fall under this definition. Pre-packed foods are required by law to declare specific allergenic foods as ingredients on the label regardless of the level present.

[2] For the purpose of informing food business operators when samples have been taken, a brand owner is considered to be the person or company who 'owns' the product. It is a legal requirement that the brand owner is listed on the label however; this is not always the case. Where information is lacking and unobtainable the vendor (by default) is considered the brand owner.

The Head Office of retail chains will also be sent a list of all the samples purchased from their stores.

What will happen to the sample?

The sample will be sent to a testing laboratory at Reading Scientific Services Limited (RSSL) where it will be analysed for the presence and level of one or more of the following four major food allergens: milk, gluten, peanut and hazelnut. The results will then be sent to the FSA. Where possible, a portion of the sample will be kept in case the manufacturer of the product asks for further independent analyses.

How will samples be tested?

Samples will be analysed using protein based methods such as enzyme linked immuno-sorbent assays (ELISA). These will be able to detect and measure the level of allergenic protein in the food sample.

What will happen if the results are unusual?

Any unusual results will be sent to the FSA for review and risk assessment, the FSA will contact the brand owner to explain what happens next. If there is an urgent need to withdraw or recall the product because it is in breach of the law and/or to protect public health, the brand owner will be notified as soon as possible of the action required. This survey is being carried out to collect information rather than for enforcement purposes but if the results show a hazard to health, or breach relevant legal requirements, your local authority will be informed and they may wish to take further samples as part of an investigation.

What will happen at the end of the survey?

At the end of the survey, a final technical report will be published on the FSA's web site (http://www.food.gov.uk). You can email the FSA at: Nathalie.Shapiro@foodstandards.gsi.gov.uk to be informed when this has happened and receive a link to the report.

How can I find out more?

Further information about the survey can be found at: http://www.food.gov.uk/science/research/foodcomponentsresearch/allergyresearch/t07programme/t07projectlist/fs241038/. You can also call the FSA on: +44 (0) 207 276 8536, and tell them that a sample was taken from your establishment for the following survey:

SURVEY OF ALLERGEN ADVISORY LABELLING AND ALLERGEN CONTENT OF UK RETAIL PRE-PACKED PROCESSED FOODS

The FSA would like to thank you for your cooperation.

2.2 Letter to Interested Parties

Food Standards Agency
food.gov.uk

rssl
science with service

Letter sent by email

Date: 16th July 2012

Dear Interested Parties,

FOOD STANDARDS AGENCY SURVEY OF ALLERGEN ADVISORY LABELLING AND ALLERGEN CONTENT OF UK RETAIL PRE-PACKED PROCESSED FOODS

The Food Standards Agency (FSA) carries out a wide-ranging programme of food surveys to:

- check that food is safe for consumers and that they are not being misled
- collect information on the composition of food
- help develop the FSA's polices on food safety
- check if current laws, codes of practice and other measures are working.

Details of current survey programmes can be found at:
http://www.food.gov.uk/science/surveillance/

The FSA is currently undertaking a survey to investigate allergen advisory labelling and allergen content of pre-packed processed foods sold in the UK. We are writing this letter to inform food business operators that food samples will be purchased from a range of retail outlets across the UK including major and smaller national supermarkets as well as independent retailers for the purpose of this survey.

Sampling is due to begin the week commencing the 16th July 2012 and will last for approximately 8 months. The survey is being managed by Reading Scientific Services (RSSL) with sampling undertaken by Ventress Technical Ltd. The analysis of samples will be carried out by RSSL.

A broad range of pre-packed processed foods with and without allergen advisory labelling will be sampled and analytically tested for the unintentional presence and level of one or more of the following four major food allergens: milk, cereals containing gluten, peanut and hazelnut. The survey will examine the different types of advisory statements used on pre-packed foods and

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125 Kingsway
London WC2B 6NH
T 020 7276 8536
E Nathalie.Shapiro@foodstandards.gsi.gov.uk

compare the use of these phrases to the level of allergens present. It will also examine whether the suggested labelling statements set out in the FSA's Best Practice Guidance are being used by food business operators. It is anticipated that the results of this survey will help to inform the development of risk based proportionate allergen management thresholds (known as action levels).

Further information about the survey can be found at: http://www.food.gov.uk/science/research/foodcomponentsresearch/allergyresearch/t07programme/t07projectlist/fs241038/ and in Annex 1 below.

We appreciate your cooperation during the sampling period of the survey.

Please do not hesitate to contact me if you have any queries.

Yours faithfully,

Nathalie Shapiro
Project Officer – Food Allergy Branch

email: Nathalie.Shapiro@foodstandards.gsi.gov.uk
direct telephone: 020 7276 8536

cc **Sue Hattersley (Head of the Food Allergy Branch)**
Chun-Han Chan (Food Allergy Legislation and Risk Assessment Manager)
Sarah Hardy (Food Allergy Research Programme Manager)

ANNEX 1: FURTHER INFORMATION ABOUT THE SURVEY

Why has the FSA chosen to purchase samples from your retail outlet?

Your retail outlet has been selected at random because it is one of the places where people buy the type of samples the FSA are looking at in their current survey of allergen advisory labelling.

How will I know which products have been taken?

A list of all the samples taken for the survey will be held by the FSA. Details of the samples purchased from an establishment will be sent to the brand owner[1] as soon as practicable. The Head Office of retail chains will also be informed when samples have been purchased.

What will happen to the sample?

The sample will be sent to RSSL where it will be analysed for the unintentional presence and level of one or more of the following four major food allergens: milk, cereals containing gluten, peanut and hazelnut. The results will then be sent to the FSA. Where possible, a portion of the sample will be kept in case the manufacturer of the product asks for further independent analyses.

How will samples be tested?

Samples will be analysed using protein based methods such as enzyme linked immuno-sorbent assays (ELISA). These will be able to detect and measure the level of allergenic protein in the food sample.

What will happen if the results are unusual?

Any unusual results will be sent to the FSA for review and risk assessment, the FSA will contact the brand owner to explain what happens next. If there is an urgent need to withdraw or recall the product because it is in breach of the law and/or to protect public health, the brand owner will be notified as soon as possible of the action required. This survey is being carried out to

[1] For the purpose of informing food business operators when samples have been taken, a brand owner is considered to be the person or company who 'owns' the product. It is a legal requirement that the brand owner is listed on the label however; this is not always the case. Where information is lacking and unobtainable the vendor (by default) is considered the brand owner.

collect information rather than for enforcement purposes but if the results show a hazard to health, or breach relevant legal requirements, your local authority will be informed and they may wish to take further samples as part of an investigation.

What will happen at the end of the survey?

At the end of the survey, a final technical report will be published on the FSA's web site (http://www.food.gov.uk). You can email the FSA at Nathalie.Shapiro@foodstandards.gsi.co.uk to be informed when this has happened and receive a link to the report.

3 Neogen Biokits Peanut Investigation
3.1 Neogen's Final Investigation Report

Selected One of Forbes' Top 200 Small Companies

November 18, 2013

To whom it may concern:

I wanted to take this opportunity to personally thank everyone from RSSL and the Food Standards Agency for bringing the matter of soy crossreactivity in the Biokit Peanut assay to the attention of Neogen's Technical Service and Food Safety management team. Having had an ISO 9001 accredited quality system in place for more than 10 years, we certainly pride ourselves in fully investigating all issues related to product performance. While it is not our policy to share the specifics of our investigation, these measures are most certainly taken. I have however based on many of your suggestions, modified the enclosed report. Please note however that this product is not frequently manufactured therefore there are a very limited number of lot numbers still within expiry that are available for such an investigation and no remaining original antibody inventory in which to compare to. Our performance complaint system also shows no reported issues on this product related to potential crossreactivity.

At this time Neogen has chosen not to take measures to further alter the performance of the Biokit Peanut assay and place our full attention to the continued support of the Veratox line. The Biokit Peanut kit insert will be amended to reflect the discovered crossreactivity and we now consider this matter closed.

Best regards,

Anthony J. Lupo
Director of Technical Service
Neogen Corporation
tlupo@neogen.com

800-234-5333 x4459

Biokits Peanut Cross-reactivity to Soy Investigation

It was brought to the attention of Neogen Corporation by RSSL that the Biokits Peanut test kit (Product # 902048Q) was potentially reporting false detections showing 1-10 ppm positive results with soy containing products believed to not contain peanut. Acquired by Neogen from Tepnel in 2009 with several lots of qualified antibody, this product line has been successfully manufactured by Neogen for the past 4 years. Further analysis using the Veratox for Peanut test kit (Neogen's primary allergen platform Cat#8430) confirm the absence of peanut in these samples. Upon further review, the effects appear to coincide with a change to a different batch of qualified Tepnel supplied antibody that occurred in April 2012 in lots 145012 and newer. Having no reason to suspect previously qualified reagents, a full new antibody characterization would not have been conducted when the change was made and we thank you for bringing the matter to our attention.

This document summarizes the investigation of the matter in addition to providing further detail about Neogen quality procedures related to this and all kits.

Neogen Antibody Characterization

When making a change to an immunodiagnostic test in which a new antibody is used, or a new version of the same antibody is used, three pilot lots are produced using the new antibody. These lots are all tested using existing quality protocols. When all pilot lots have passed full QC inspections Manufacturing contacts the Technical Service group to schedule a validation test. The scope of the study is discussed and a determination is made as to what array of samples and concentrations are to be tested to thoroughly evaluate the product. The three pilot lots are tested in Technical Service alongside the original version of the product. The new antibody must show equivalent or improved performance to the existing product which includes a full cross reactivity panel to pass. (Appendix A) For validation of the method, depending upon the change, the Validations lab will also be engaged. The testing performed by this group is geared towards examining the robustness of the new test. Multiple operators over multiple days will evaluate the product using a smaller array of samples on all three pilot lots as well as a control lot. The compiled data is evaluated for robustness and reproducibility. The pilot lots must be equivalent or superior to the control lot to pass. Due to the prequalified status of these reagents by Tepnel, this standard antibody characterization was not performed however in the future all previously qualified antibody fractions will be requalified.

Biokits Peanut Quality Specifications

All bulk reagents in the Peanut Biokit (prior to dispensing) are tested to ensure all specifications are met prior to dispensing. 10 replicates of the 0 control, 6 replicates of the standards and duplicates of the positive/negative samples included in the kit are tested on a random plate from the lot. The following specs must be met in order to approve the kit for dispensing/finishing:

Parameter	Specification
Mean OD 0ppm	<0.185
Mean OD standard 1 (1ppm)	≥1.75 x OD of
Mean OD standard 4 (10ppm)	>1.2
Mean OD standard 5 (20ppm)	1.8-2.6
Biscuit Crumb OD	≤0.262
Peanut Spike, ppm	3.0-7.0
R-squared: (correlation coefficient)	>0.961

For final testing, 1 complete, random kit is supplied to QC and tested/approved as above. In addition a packaging inspection also occurs to ensure all components are accounted for and properly labeled. All specifications were either unchanged or more stringent than original Tepnel QC specifications.

Determination of % soy needed to cause effect:
Lansing Technical Service transferred BioKit Peanut Test Kit lot # 192305 and added soy flour to peanut free mixture at the following levels: 0, 1, 10, 25, and 50%.

Results:

	BioKit Peanut	
% Soy	Raw data ppm	Avg ppm
0	0.1	0.1
	0.3	
	0.0	
1	1.2	1.3
	1.3	
	1.5	
10	2.2	2.1
	2.2	
	2.0	
25	2.1	2.0
	2.0	
	2.0	
50	2.1	2.1
	2.1	
	2.2	

Upon an internal investigation using Lot #192305, all negative samples will in fact be truly negative however a low level positive result could potentially be the result of this effect in soy levels greater than or near 1%. Although incremental dosing was not performed between zero and 1% soy, it can be inferred that the initial effects are observed near 1% due to the ppm response being so close to the kit limit of quantitation although additional data between 0 and 1% would be needed to pinpoint this if determined critical. A saturation affect appears to occur as there is no measurable difference between the effects observed at 10% and the effects observed at 50%. It can then be concluded that levels above the highest observable positives are in fact true peanut reactivity.

Soy ingredient analysis on Biokits and Veratox Peanut Assays:

In an effort to determine if all sources of soy caused this reaction, Ayr Technical Service analyzed several lots of the test kit against various sources of soy including soy flour, soy bean meal, soy protein isolate, and soy milk as well as lot to lot comparisons.

Veratox Peanut Lot 191063									
Soya Flour (RSSL)	O.D	Result (ppm)			**Soya bean Meal (RSSL)**	O.D	Result (ppm)		
Control 1	0.519	0.000			Control 1	0.524	0.000		
Control 2	0.659	2.500			Control 2	0.692	2.500		
Control 3	0.845	5.000			Control 3	0.836	5.000		
Control 4	1.133	10.000			Control 4	1.132	10.000		
Control 5	1.769	25.000			Control 5	1.666	25.000		
Soya Flour 1:25 extraction ratio	0.501	0.000	BLOQ		Soya bean meal 1:25 extraction ratio	0.479	0.000	BLOQ	
Soya Flour 1:25 extraction ratio	0.504	0.000	BLOQ		Soya bean meal 1:25 extraction ratio	0.479	0.000	BLOQ	
Soya Flour 1:10 extraction ratio	0.561	0.000	BLOQ		Soya Bean meal 1:10 extraction ratio	0.521	0.000	BLOQ	
Soya Flour 1:10 extraction ratio	0.546	0.000	BLOQ		Soya Bean meal 1:10 extraction ratio	0.492	0.000	BLOQ	
NB: the 1:10 extraction ratio was chosen to bring the range of quantification of the Veratox kit from 2.5ppm-25ppm to 1ppm-10ppm (alligned with Biokits Peanut)									
Biokits Peanut Lot 145016									
Soya Flour and Bean Meal									
	Mean O.D	Result (ppm)							
Control 1	0.104	0.000							
Control 2	0.361	1.000							
Control 3	0.449	2.000							
Control 4	0.752	5.000							
Control 5	1.128	10.000							
Control 6	1.659	20.000							
Kit assay control	0.674	4.030							
Soya Flour (RSSL)	0.582	3.080							
Soya Bean meal (RSSL)	0.308	0.830	BLOQ						
Soy Protein Isolate	0.138	0.050	BLOQ						

Soy Milk Analysis on Biokits and Veratox Peanut Assays:

Veratox Peanut Lot 191063			
Soya Milk (full fat)			
	O.D	Result (ppm)	
Control 1	0.470	0.000	
Control 2	0.692	2.500	
Control 3	0.897	5.000	
Control 4	1.205	10.000	
Control 5	1.871	25.000	
Soya Milk 1:25 extraction ratio	0.483	0.200	
Soya Milk 1:25 extraction ratio	0.480	0.100	
Soya Milk 1:25 extraction ratio	0.496	0.300	
Soya Milk 1:10 extraction ratio	0.443	0.000	
Soya Milk 1:10 extraction ratio	0.444	0.000	
Soya Milk 1:10 extraction ratio	0.457	0.000	
NB: the 1:10 extraction ratio was chosen to bring the range of quantification of the Veratox kit from 2.5ppm-25ppm to 1ppm-10ppm (alligned with Biokits Peanut)			
Biokits Peanut Lot 145016			
Soya Milk (full fat)			
	Mean O.D	Result (ppm)	
Control 1	0.084	0.000	
Control 2	0.273	1.000	
Control 3	0.330	2.000	
Control 4	0.613	5.000	
Control 5	0.880	10.000	
Control 6	1.054	20.000	
Kit assay control	0.515	3.580	
Soya Milk (standard extraction)	0.155	0.430	BLOQ
Soya Milk (standard extraction)	0.143	0.350	BLOQ

Soy Flour analysis on various test kit lots:

Peanut Biokits			
Biokits Peanut Lot 145016			
Soya Flour (four extracts comparison)			
	Mean 0.D	Result (ppm)	
Control 1	0.148	0.000	
Control 2	0.237	1.000	
Control 3	0.388	2.000	
Control 4	0.742	5.000	
Control 5	1.285	10.000	
Control 6	2.100	20.000	
Kit assay control	0.716	4.770	
Soya Flour 1	1.351	10.670	
Soya Flour 2	1.372	10.890	
Soya Flour 3	1.483	12.080	
Soya Flour 4	1.412	11.310	
Biokits Peanut Lot 188851			
Soya Flour (four extracts comparison)			
	Mean 0.D	Result (ppm)	
Control 1	0.148	0.000	
Control 2	0.300	1.000	
Control 3	0.490	2.000	
Control 4	0.794	5.000	
Control 5	1.291	10.000	
Control 6	1.853	20.000	
Kit assay control	0.609	3.140	
Soya Flour 1	1.080	7.660	
Soya Flour 2	1.012	6.900	
Soya Flour 3	0.977	6.510	
Soya Flour 4	0.884	5.580	

Based on the provided data, samples reporting over 12 ppm are not suspect at this time. It can also be stated that soy milk, soy bean meal, and soy protein isolates have not reproduced the effects observed by RSSL on these matrices. The Veratox Peanut assay has also not shown any of the effects.

In addition an exhaustive evaluation of the performance complaint systems uncovered no additional issues related to Peanut Biokit at this time. All current customers have been notified and no additional issues have arisen.

Summary:

The most immediate path forward is to offer analytic support services on such low level positive results to confirm using the Veratox Peanut assay. An additional immediately available solution would be to provide all relevant validation data to implement the Veratox Peanut assay at RSSL. While further work could be done to investigate and characterize the reagents of the Biokits assay, this is would be a longer term endeavor and would involve significant rework of the Biokits assay. Any changes to the assay (including antibody or how antibody is prepared) will result in a full review of the overall profile of the commercial kit including specificity and sensitivity and would elicit a complete reevaluation of the standard cross-reactivity panel found in Appendix A. These options have been discussed however Neogen has chosen to take no action other than revising the test kit insert at this time. Based on Neogen's full investigation, negatives can be assumed to be negative however positives below 12 ppm should be considered suspect if the sample is suspected to contain soy.

Anthony J. Lupo
Director of Technical Service
Neogen Corporation

November 18, 2013
Date

Appendix A:

CROSS-REACTIVITY

All of the following have no cross-reactivity at 100% with the Veratox for Peanut Allergen test.

No.	Commodity	No.	Commodity
1	Almond	17	Oat
2	Barley	18	Pecan
3	Bovine gelatin	19	Pine nut kernel
4	Brazil	20	Pistachio
5	Buckwheat	21	Poppy
6	Cashew	22	Porcine gelatin
7	Chestnut	23	Pumpkin
8	Chick pea	24	Rice
9	Cocoa	25	Rye
10	Coconut	26	Sesame
11	Corn	27	Skim milk powder
12	Green pea	28	Soybean (Dosoy soya flour)
13	Hazelnut	29	Sunflower
14	Lecithin	30	Walnut
15	Lima (butter) bean	31	Wheat
16	Macadamia	32	Wheat gluten

All values reported as < Limit of quantitation for pure substance are considered not to cross react

3.2 Letter from Neogen to RSSL

NEOGEN® CORPORATION

Selected One of Forbes' Top 200 Small Companies

September 12, 2013

Reading Scientific Services LLC
Reading, United Kingdom

Dear Sir or Madam,

It was brought to the attention of Neogen Corporation by RSSL that lots# 145012 and 145013 of the Biokits Peanut test kit (Product # 902048Q) were potentially showing false detections showing 1-10 ppm positive results on soy containing products believed to not contain peanut. Acquired by Neogen from Tepnel in 2009 with several lots of qualified antibody, this product line has been successfully manufactured by Neogen for the past 4 years.

Further analysis using the Veratox test kit (Neogen's primary allergen platform) confirm the absence of peanut in these samples. Upon further review, the effects appear to coincide with a change to a different batch of qualified Tepnel supplied antibody. Having no reason to suspect previously qualified reagents, this would not have been investigated when the change was made and we thank you for bringing the matter to our attention.

Upon an internal investigation using Lot #192305, all negative samples will in fact be truly negative however a low level positive result could potentially be the result of this effect. However based on enclosed data, samples reporting over 2 ppm are not suspect at this time it is understood that at RSSL values up to 12 ppm could be questionable.

The most immediate path forward is to offer analytic support services on such low level positive results to confirm using Veratox. An additional immediately available solution would be to provide all relevant validation data to implement the Veratox assay at RSSL. While further work could be done to investigate and characterize the reagents of the Biokits assay, this is would be a longer term endeavor.

Please feel free to contact me should you have any further questions.

Best regards,

Anthony J. Lupo
Director of Technical Service
Neogen Corporation

800-234-5333 x4459

Enclosure

620 Lesher Place, Lansing MI 48912
517/372-9200 • 800/234-5333 • fax: 517/372-0108
neogen-info@neogen.com • www.neogen.com

3.3 Letter from Neogen to Interested Parties

European Headquarters of Neogen Corporation
The Dairy School Auchincruive Ayr KA6 5HW Scotland UK
Tel: +44 (0) 1292 525 600 Fax: +44 (0) 01292 525 601
E-mail: info@neogeneurope.com

10th October 2013

Dear Valued Customer,

We would like to advise you that our BioKits Peanut Assay may show false positive detections in the low ppm range on products containing significant amounts of unprocessed soy flour. This was reported by a customer and while we are investigating this matter we wish to draw your attention to it.

The sensitivity and reliability of the test kit to detect peanut are not affected.

Further analysis on known negative soy flour samples was carried out using Neogen's alternative peanut assay, the Veratox Peanut kit, on both standard and high sensitivity extractions. These results were below the limit of quantification.

The effect (cross reaction with unprocessed soy) appears to be limited to a previously qualified batch of antibody that was transferred to routine manufacturing. We are looking at measures to reduce the low level response on unprocessed soy flour. However, we would recommend that positive results from products containing soy flour are confirmed using an alternative method.

We would be happy to supply Veratox Peanut to assist in this work, and are happy to offer additional technical support and product testing at Neogen Europe, as appropriate.

We would welcome any additional comments or feedback regarding performance of the BioKits Peanut. If you have any concerns or would like any additional information, please do not hesitate to contact me.

Yours sincerely,

Paulini Titchene

Business Development Executive – Allergens and Speciation