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

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, through 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: approximately **40 minutes** (5 samples) Test incubation time: **75 minutes**
Specificity:	The polycolonal antibody specifically detects Conarachin (Ara h1)
Cross-reactivity:	**This test cross-reacts with soya.** Of a large panel of other 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	✓
Biscuit crumb		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)

1. Sodium chloride (Neogen item 7725G)
2. Tris (hydroxymethyl) methylamine (Sigma 93349)
3. 1M hydrochloric acid (Fisher 393260)
4. Teleost gelatin (Sigma G7765)

Equipment

1. Pestle and mortar, Stomacher or suitable grinder/mill that can be easily cleaned after use to eliminate the possibility of cross-contamination between samples
2. Miscellaneous laboratory plastic and/or glassware, including measuring cylinders, pipettes, disposable Pasteur pipettes, plate seals and containers suitable for food extracts
3. pH Meter
4. Water bath capable of maintaining 60 ± 2°C

5. Wrist action or similar shaker
6. Centrifuge and appropriate centrifuge/microfuge tubes for clarifying sample extract
7. Micropipette(s), 50–100 µL (Neogen item 9276)
8. Disposable tips for micropippettes, 50–100 µL (Neogen items 9407, 9410, 9417)
9. Microwell washer (e.g., NUNC Immuno Wash 8) or wash bottle
10. 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 mg of peanut material can be detected in approximately 1 kg of food. However, such a limit of detection assumes food samples can be adequately sampled prior to extraction and also that they can be rendered truly homogeneous.

It is strongly recommended the user note the order in which samples are processed so 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 also can be used for BioKits egg and sesame assay kits.

Prepare high salt Tris extraction buffer for the extraction of food samples. Amounts quoted are for 1.5 L — 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 1500 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 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 mL) 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 (a flour or paste is preferable) 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 biscuit crumb with 10 µL/g (e.g., 50 µL for 5 g) of Peanut Control. Mix well and set aside for at least **30 minutes** while preparing other reagents and 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 accidentally is added 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 the portion 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 (Neogen item 901042J). This kit can be used in conjunction with the BioKits Peanut Assay kit for the determination of peanut contamination levels in the environment.

TEST PROCEDURE

For practice assays, it is recommended 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 quality control 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 four more times (five 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 Conjugate. 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 (e.g., salinity, acidity, etc.), treatment of foodstuffs during processing (e.g., heat, pressure, etc.), difficulty of obtaining complete homogeneity during extraction, and the reactivity of different sources of peanut material means 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 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 that 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 one 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 (e.g., room) temperatures should be minimized.

Peanut plate: The 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 Conjugate. Such imprecision is an indication of a problem during the performance of the assay, which may be invalid and need to be repeated.

A validation report for BioKits Peanut Assay is available upon request.

CUSTOMER SERVICE

Neogen Customer Assistance and Technical Services can be reached by using the contact information on the back of this booklet. Training on this product, and all Neogen test kits, is available.

SDS INFORMATION AVAILABLE

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

TERMS AND CONDITIONS

For Neogen's full terms and conditions, please visit www.neogen.com/Corporate/termsconditions.html.

WARRANTY

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TESTING KITS AVAILABLE FROM NEOGEN

Natural toxins
- Aflatoxin, DON, ochratoxin, zearalenone, T-2/HT-2 toxins, fumonisin, histamine

Foodborne bacteria
- *E. coli* O157:H7, *Salmonella*, *Listeria*, *Listeria monocytogenes, Campylobacter, Staphylococcus aureus, Salmonella enteritidis*

Sanitation
- ATP, yeast and mold, total plate count, generic *E. coli* and total coliforms, protein residues

Food allergens
- Almonds, crustaceans, eggs, gliadin, hazelnut, milk, mustard, peanuts, sesame, soy, walnuts, multi-treenut

Genetic modification
- CP4 (Roundup Ready®)

Ruminant by-products
- Meat and bone meal, feed

Species Identification
- Raw and cooked meat samples

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