AgraQuant® Peanut Assay (1 - 40 ppm)

Order #: COKAL0148

Intended Use

The AgraQuant® Peanut Assay is a sandwich enzyme-linked immunosorbent assay (ELISA) that determines a quantitative level of the presence of peanut in food.

The AgraQuant® Peanut Assay represents a highly sensitive detection system designed for the quantification of peanut residues in a variety of food products.

Peanut

The peanut or groundnut (*Arachis hypogaea*) is a legume and produces nut-like fruits within protective shells. Peanuts are a highly nutritious source food for man and animals, containing high amounts of oil and storage proteins (about 25%), many of which, particularly Arachins and Conarachins, are allergenic. For people with a peanut allergy, even minor exposure in the nano-gram range can cause from mild skin rashes to fatal anaphylactic shock.

People who are allergic to peanuts must strictly avoid the consumption of peanuts or peanut-containing food. Cross contamination during the production process often occurs so peanut residues in food cannot be excluded. Since processed peanut materials, such as flours, defatted/deflavoured products etc. can make detection in foods difficult, extremely sensitive detection systems are required.

Assay Principles

The AgraQuant® Peanut Assay is a sandwich enzyme-linked immunosorbent assay (ELISA). Peanut proteins are extracted from a sample with an extraction buffer. Antibodies directed against peanut proteins are pre-coated on the surface of a microwell. The extracted sample or standards are applied to the wells and peanut proteins bind to the antibodies. After a washing step an enzyme-conjugated antibody specific to peanut proteins is applied to the well and incubated. After a second washing step, an enzyme substrate is added and blue color develops. The intensity of the color is directly proportional to the concentration of peanut in the sample or standard. A stop solution is then added which changes the color from blue to yellow. The microwells are measured optically using a microwell reader with a primary absorbance filter of 450nm (OD_{450}). The optical densities of the samples are compared to the OD's of the standards and an interpretative result is determined.

Precautions

1. Store reagents at 2-8°C (35-46°F) when not in use, and do not use beyond the expiration date.
2. Adhere to incubation times stated in the procedure. Use of incubation times other than those specified may give inaccurate results.
3. Due to high risk of cross contamination all used instruments must be cleaned thoroughly before sample preparation.
4. The Stop Solution contains acid. Avoid contact with skin or eyes. If exposed, flush with water.
5. Wear protective gloves and safety glasses when using the kit.
6. Dispose of all materials, containers and devices appropriately after use.

keydiagnostics
T: 02 8212 4074 F: 02 9423 6992
info@keydiagnostics.com.au
www.keydiagnostics.com.au
PO Box 1038, Gymea, NSW, 2227

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Solution Preparation

Extraction buffer

Dilute extraction buffer concentrate 1:10 with distilled water (e.g. add 10 mL of concentrated extraction buffer to 90 mL distilled water). Store at 4°C. The diluted buffer is stable for one week.

Wash buffer

If during the cold storage crystals precipitate, the concentrate should be warmed up until they are dissolved. Dilute wash buffer concentrate1:10 with distilled water (e.g. add 10 mL of concentrated wash buffer to 90 mL distilled water). Store at 4°C. The diluted wash buffer is stable for four weeks.

Procedure

Sample Preparation / Extraction

1. Obtain a representative sample and homogenize a minimum of 5 g in a mortar or blender.
2. Weigh out 1 g of homogenized sample and mix with 20 mL of pre-diluted extraction buffer and vortex.
3. Incubate the suspension for 15 minutes in a preheated water bath at 60°C and vigorously shake every two minutes.
4. Centrifuge samples for 10 minutes at 2000 g to obtain a clear supernatant. If there are still particles in the supernatant filter the supernatant and collect filtrate. If centrifuge is not available filter the extract by using filter paper and then collect the filtrate.
5. Samples are ready for testing. Apply 100 µL of particle-free solution per well. If the results of a sample are out of the range of quantitation, further dilution with the pre-diluted extraction buffer is necessary. The additional dilution must also be considered when calculating the concentration

Assay

Note: All reagents and kit components must be at room temperature 18-30°C (64-86°F) before use. It is recommended that an 8-channel pipettor be used to perform the assay. No more than 48 samples and standards total (6 test strips) should be run in one experiment when using an 8-channel pipettor.

1. Place an appropriate number of Antibody Coated Microwells in a microwell strip holder. Return unused microwells to the foil pouch with the desiccant packet and reseal pouch.
2. Using a single channel pipettor, add **100 µL of each ready-to-use standard or prepared sample** into the appropriate well. Use a fresh pipette tip for each standard or sample. **Note:** Make sure the pipette tip has been completely emptied.
3. Incubate at room temperature for **20 minutes. Note:** Do not agitate the plate to mix as it may cause well-to-well contamination.
4. Empty the contents of the microwell strips into a waste container. Wash by filling each microwell with diluted wash buffer, and then dumping the water from the microwell strips. Repeat this step 4 times for a total of 5 washes. **Note:** Take care not to dislodge the strips from the holder during the wash procedure.
5. Lay several layers of absorbent paper towels on a flat surface and tap microwell strips on towels to expel all of the residual water after the fifth wash. Dry the bottom of the microwells with a dry cloth or towel.
6. Measure the required amount of Conjugate from the green-capped bottle (~120 µL/well or 1 mL/strip) and place in a separate container (e.g. reagent boat when using the 8-channel pipettor). Using an 8-channel pipette, dispense **100 µL of Conjugate** into each well.
7. Incubate at room temperature for **20 minutes. Note:** Do not agitate the plate to mix as it may cause well-to-well contamination.
8. Empty the contents of the microwell strips into a waste container. Wash by filling each microwell with diluted wash buffer, and then dumping the water from the microwell strips. Repeat this step 4 times for a total of 5 washes. **Note:** Take care not to dislodge the strips from the holder during the wash procedure.

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9. Lay several layers of absorbent paper towels on a flat surface and tap microwell strips on towels to expel all of the residual water after the fifth wash. Dry the bottom of the microwells with a dry cloth or towel.
10. Measure the required amount of Substrate from the blue-capped bottle (~120 µL/well or 1 mL/strip) and dispense into a separate container (e.g. reagent boat for an 8-channel pipettor). Pipette **100 µL of the Substrate** into each microwell using an 8-channel pipettor. Incubate at room temperature for **20 minutes** in the dark (e.g. cover completely, or CAREFULLY place in a cupboard or drawer).
11. Measure the required amount of Stop Solution from the red-capped bottle (~120 µL/well or 1 mL/strip) and dispense into a separate container (e.g. reagent boat for an 8-channel pipettor). Pipette **100 µL of Stop Solution** into each microwell using an 8-channel pipettor. The color should change from blue to yellow.
12. Read the strips with a microwell reader using a 450 nm filter. Record OD readings for each microwell. **Note:** Air bubbles should be eliminated prior to reading strips as they may affect analytical results.

Additional Notes: Do not return unused reagents to their original bottles. Carefully keep track of the position of Samples and Standards during the assay. Do not mix the assay microwells by shaking at any time during test.

Interpretation of the Results

Using either the unmodified OD values or the OD values expressed as a percentage of the OD of the 40 ppm standard, construct a dose-response curve using the five standards. Since the amount of peanut in each standard is known, the unknowns can be measured by interpolation from this standard curve. Results can also be easily calculated using the Romer Labs® spreadsheet that is provided (free of charge) upon request. An OD value of less than 1.1 absorbance units for 40 ppm standard may indicate deterioration of reagents.

If a sample contains peanut levels higher than the highest standard (>40 ppm), the sample extract should be further diluted in extraction buffer such that the diluted sample results are in the range of 1 - 40 ppm and reanalyzed to obtain accurate results. The dilution factor must be included when the final result is calculated.

Performance Characteristics

Limit of detection:	0.1 ppm (Determined by the average values of 10 buffer blanks plus 3 standard deviations)
Limit of quantitation:	1 ppm (Described as the lowest concentration point on the calibration curve that this test can reliably detect peanut).
Range of quantitation:	1 – 40 ppm (For quantitation of samples above 40 ppm samples should be diluted such that the diluted sample results are in the range of 1 - 40 ppm).
Cross Reactivity to:	Fenugreek: 0.00015% Gum Arabic: 0.003% Soy flour: 0.0002%

Materials Supplied With Kit

- 48 antibody coated microwells (6 eight-well strips) in a microwell holder (sealed in a foil pouch)
- 5 vials of 4 mL of each peanut standard (0, 1, 4, 10 and 40 ppm)
- 1 bottle of 7.5 mL of conjugate (green-capped bottle)
- 1 bottle of 7.5 mL of substrate solution (blue-capped bottle)
- 1 bottle of 7.5 mL of stop solution (red-capped bottle)
- 1 bottle of 120 mL of 10x concentrated extraction solution
- 1 bottle of 60 mL of 10x concentrated wash buffer

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Materials Required But Not Provided With Kit

Extraction Procedure

- *EQOLE1025: Blender or a tightly sealing jar with lid, or mortar
- *EQOLE1010: Balance, 400 g
- *EQOLE1050: Graduated cylinder: 100mL
- Distilled or de-ionized water for diluting concentrated buffers
- Container with a minimum 20mL capacity
- Water bath 60°C
- Centrifuge, Microcentrifuge or Filter and Funnel
- Centrifuge tubes

Assay Procedure

- *8-channel and single channel pipettors capable of pipetting 100µL with tips
- *EQOLE1300: Timer
- *COKAD1150: Wash bottle
- Distilled or de-ionized water
- Absorbent paper towels
- *3 reagent boats for use as reagent containers for an 8-channel pipettor
- *Microwell reader with a 450nm filter, (e.g. Stat Fax® 303 Plus manufactured by Awareness Technology Inc. or the EL301 manufactured by BIO-TEK® Instruments, Inc.) or equivalent.

*Items available from Romer Labs, Inc.® - Americas Division

For further information please contact:

Technical Services
Romer Labs UK Ltd.
Block 5, The Heath Business & Technical Park,
Runcorn, Cheshire
WA7 4QX, UK

Tel: +44 845 519 50 10

Web: http://www.romerlabs.com
Email: enquiry@romerlabs.com

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