



AgraQuant[®] Pistachio Assay (1 – 40 ppm)



Order #: COKAL2748

Intended Use

The AgraQuant[®] Pistachio Assay is a sandwich enzyme-linked immunosorbent assay (ELISA) that quantitates the level of Pistachio in food.

The AgraQuant[®] Pistachio Assay is designed for laboratory use with a range of samples including raw and processed foods, rinse waters and swabs.

Pistachio

Pistachio (*Pistacia vera*) belongs to the family of *Anacardiaceae*. At approximately 21%, the protein fraction in pistachio seed is very high. Some of these proteins, for example the 2S albumin Pis v 1, the 11S globulin Pis v 2 or the 7S vicillin Pis v 3 are known to be allergenic. Many of the proteins are heat stable making them resistant to different production processes. For this reason pistachio represents an important food allergen.

For pistachio-allergic persons, hidden pistachio allergens in food are a critical problem. Very low amounts of pistachio can cause allergic reactions, which may lead to anaphylactic shock in severe cases. Because of this, pistachio-allergic persons must strictly avoid the consumption of pistachio-containing food. Cross-contamination, mostly as a consequence of the production process, is often noticed. This explains why in many cases the existence of pistachio residues in food can not be excluded. For this reason sensitive detection systems for pistachio residues in foodstuffs are required.

Assay Principles

The AgraQuant[®] Pistachio Assay is a sandwich enzyme-linked immunosorbent assay (ELISA). Pistachio proteins are extracted from a sample using an extraction buffer. Antibodies directed against Pistachio proteins are pre-coated on the surface of a microwell. The extracted sample or standards are applied to the wells and the Pistachio proteins bind to the antibodies. After a washing step, an enzyme-conjugated antibody specific to Pistachio proteins is applied to the well and incubated. After a second washing step, an enzyme substrate is added and blue colour develops. The intensity of the colour is directly proportional to the concentration of Pistachio in the sample or standard. A stop solution is then added which changes the colour from blue to yellow. The microwells are measured optically using a microwell reader with a primary absorbance filter of 450nm (OD₄₅₀). The optical densities of the samples are compared to the OD's of the standards and an interpolated result is determined.

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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 have to 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.

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 Pistachio 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

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: 100 mL
- Distilled or de-ionised water for diluting concentrated buffers
- Container with a minimum 20 mL capacity
- Water bath 60°C
- Flask Shaker (Stuart SF1 or equivalent)
- Centrifuge, Microcentrifuge or Filter and Funnel
- Centrifuge tubes
- Gelatin from cold water fish skin : Sigma G7765 (for chocolate extraction)

Assay Procedure

- *8-channel and single channel pipettors capable of pipetting 100µL with tips
- *EQOLE1300: Timer
- *COKAD1150: Wash bottle
- Distilled or de-ionised water
- Absorbent paper towels
- *3 reagent boats for use as reagent containers for an 8-channel pipettor
- *Microwell reader with a 450 nm filter
- Optional: *Transfer wells for application of samples and kit standards

*Items available from Romer Labs



Solution preparation

Extraction buffer

Dilute extraction buffer concentrate 1:10 with distilled water (e.g. add 10mL of concentrated extraction buffer to 90mL distilled water). Heat to 60°C using a water bath. The diluted buffer is stable for one week if stored at 4°C.

Wash buffer

If during the cold storage crystals precipitate, the concentrate should be warmed up until they are dissolved. Dilute wash buffer concentrate 1:10 with distilled water (e.g. add 10mL of concentrated wash buffer to 90mL distilled water). Store at 4°C. The diluted wash buffer is stable for four weeks.

Pistachio Standards

Pistachio Standards (0, 1, 4, 10, 40 ppm of Pistachio) 5 vials with 4.0mL, ready to use.

Procedure

Sample Preparation / Extraction

1. Obtain a representative sample and homogenise a minimum of 5 g in a mortar or blender.
2. Weigh out 1 g of homogenised sample and mix with 20 mL of pre-diluted, heated extraction buffer and vortex.
3. Shake the suspension for 15 minutes.
4. Centrifuge samples for 10 minutes at 2000 g to obtain a clear aqueous layer between the particulate and fat layers. If there are still particles in the supernatant filter the supernatant and collect filtrate. If a 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

For the preparation of environmental swab samples the AgraQuant® Allergen Swabbing Kit is required (Product Code. COOLS0120). This kit can be used in conjunction with the AgraQuant® Pistachio Assay Kit for the determination of Pistachio contamination levels in the environment.



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 should be run in one experiment when using an 8-channel pipettor (24 when samples and standards are added in duplicate e.g. 6 test strips).

It is good laboratory practice that duplicates are run for some or all diluted extracts and standards.

Optional Transfer well method.

- 1 Place an appropriate number of transfer wells (available on request) into a microwell strip holder.
- 2 Using a single channel pipettor, add **150 µL of each diluted 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 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.
- 4 Using an 8-channel pipettor transfer **100 µL of each ready-to-use standard or prepared samples** into the corresponding Antibody Coated Microwells.

Continue to step 3 of the Standard Method below

Standard Method

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 diluted 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 emptying the buffer 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 buffer 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 (~120µL/well or 1mL/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 emptying the buffer 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.
 9. Lay several layers of absorbent paper towels on a flat surface and tap microwell strips on towels to expel all of the residual buffer 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 (~120µL/well or 1mL/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 (~120µL/well or 1mL/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 colour 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 Pistachio 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 Pistachio 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 reanalysed to obtain accurate results. The dilution factor must be included when the final result is calculated.



Pistachio content of swab samples

The Pistachio Calibration Curve can be used to provide an estimate of the Pistachio content of a swab sample using the following example:

Value for swab sample read off curve = 10 ppm

To convert into ng/ml = $10 \times 1000 = 10000$ ng/ml

As no extraction step was used (1/20 extraction) = $10000/20 = 500$ ng/ml

Performance Characteristics

Limit of detection: 0.13 ppm Pistachio

Limit of quantitation: 1.0 ppm Pistachio.

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:

Cashew	12%
Hazelnut	0.17%
Pecan	0.0005%
Sunflower seed	0.0002%
Walnut	0.0008%

No Cross Reactivity to:

Almond	Coconut	Plum
Apricot	Cod	Poppy seed
Barley	Corn	Pork meat
Bean	Cow's milk	Potato
Beef	Egg	Pumpkin seed
Bovine gelatin	Guar gum	Rice
Brazil nut	Kiwi	Rye
Carob gum	Lentil	Saccharose
Carrot	Lupin	Sesame
Celery	Macadamia	Shrimp
Cherry	Mustard	Soy
Chestnut	Oats	Soy lecithin



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For further information please contact:

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

Tel: +44 (0) 845 519 50 10

Web: <http://www.romerlabs.com>

Email: enquiry@romerlabs.com

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