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Product Manual

AgraQuant® Plus Egg ELISA test kit
Article number 10002060

Intended use
The AgraQuant® Plus Egg ELISA test kit is an immunoassay designed for the quantitative analysis of egg residues in food samples. Samples can vary from raw to processed foods, from environmental swabs to rinse water. This product is intended for laboratory use.

Performance characteristics

Limit of detection (LOD): Foodstuffs: 0.5 ppm (0.5 mg/kg) whole egg powder
Swabs: 0.05 µg/swabs whole egg powder
Rinse water: 50 µg/L whole egg powder

Limit of quantification (LOQ): Foodstuffs: 1 ppm (1 mg/kg) whole egg powder
Swabs 0.1 µg/swabs whole egg powder
Rinse water 100 µg/L whole egg powder

Range of quantification: 1 – 25 ppm (1 - 25 mg/kg) whole egg powder

Plate format: 48 wells

Assay time: sample preparation – 5-10 minutes (approx.)
total time for test – 30 minutes (approx.)

About Egg
Egg allergy is one of the most common food allergies affecting children very early in life and has a high degree of prevalence worldwide. Eggs are a very versatile ingredient and can be found in a wide range of foods: baked goods, meat products, mayonnaise and salad dressings, soups, ice cream and chocolate. Thus, they constitute a significant health risk to allergic individuals. Both egg white and yolk contain proteins known to be allergenic; however, egg white protein allergies are more common. Cross-contamination with egg during food production cannot always be ruled out, making the detection of egg proteins and their residues in food products and production lines all the more indispensable.

Product information

About the ELISA test kit

The AgraQuant® Plus Egg test kit is an enzyme-linked immunosorbent assay (ELISA) sandwich used for the detection and quantification of raw and processed eggs in food samples. This product is a very sensitive detection system and utilizes highly purified antibodies raised against whole egg powder for the quantification of egg residues and traces in a variety of food products. AgraQuant® Plus Egg can also be used to validate cleanings and to test for the presence of allergenic traces via rinse waters or environmental swab.

Storage information

Upon receipt, immediately transfer the AgraQuant® Plus Egg to refrigerated storage and keep it at 2-8°C (35-46°F) when not in use. Do not freeze. Do not use the kit beyond the expiration date indicated on the package.

Content of the kit

The AgraQuant® Plus Egg ELISA test kit contains the following items:

- 48 white-colored microwells (transfer wells, 6 eight-well strips)
- 48 antibody-coated microwells (6 eight-well strips)
- 1 microwell strips frame
- 5 white-capped vials of ready-to-use standards (0, 1, 5, 10 and 25 ppm)
- 1 bottle of 50 mL of 20X concentrated wash buffer
- 1 green-capped bottle of enzyme-conjugate solution (detection antibody)
- 1 blue-capped bottle of substrate solution
- 1 red-capped bottle of stop solution
- 1 extraction additive 1 (big beige capsule)
- 1 extraction additive 2 (small white capsule)

Materials required but not included

- Blender, mortar and pestle, or homogenizer
- Analytical balance
- Water boiler or water bath (60°C/140°F)
- Distilled or deionized water
- Centrifuge tubes (1.5 or 2 mL, 15 and 50 mL)
- Micro-centrifuge for 1.5 or 2 mL vials (speed≥8000 g) or filter and funnel
- Pipettes and tips (20-200 µL; 200-1000 µL)
- Multichannel pipette and tips (100-300 µL)
- Timer
- Plate washer or 1 L screw-cap sterile bottle for storing wash buffer
- Absorbent paper towels
- 3 reagent boats for use as reagent containers for a multichannel channel pipette
- Microwell reader with 450 and 630 nm filters

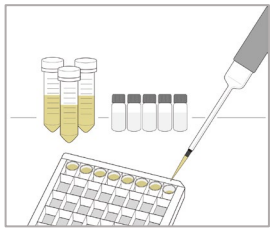
Visit **www.romerlabs.com** or get in touch with your technical sales representative to find out which of these items are also available from Romer Labs.

ELISA kit – Assay principle

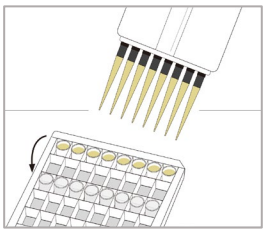
AgraQuant® Plus ELISA kits are based on solid phase sandwich ELISA technology. The kit comes with antibodies raised against the allergen of interest pre-coated onto each well of the plate that comes with the kit. During the first incubation, both samples and standards of known concentrations are added to the coated wells to allow any allergen present in the sample to bind to the immobilized antibodies.
After washing, the enzyme-conjugated solution (detection antibody) is added to the wells and binds to the allergens captured during the first incubation. This creates the so called "sandwich". After a second washing step to remove excess detection antibodies, the substrate solution is added, which results in color development. The intensity of the color developed is directly proportional to the concentration of allergen present in the samples and in the standards.
A stop solution is then added, which changes the color from blue to yellow. The absorbance of each well is then measured at 450 nm with the differential filter at 630 nm. The measurement must take place within 10 minutes after adding the stop solution.
To analyze the results, please refer to *Results analysis* at the end of this product manual.

Protocol at a glance

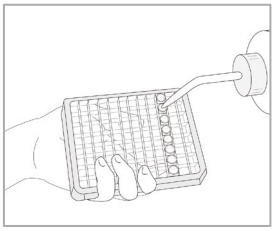
The following section gives only an overview of the ELISA procedure. Before performing the assay, carefully read through this product manual.



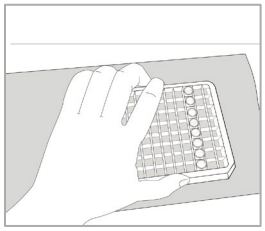
1. Dispense **samples** and **standards** into the white-colored wells (transfer wells).



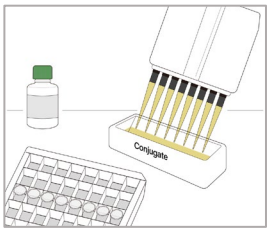
2. Transfer **samples** and **standards** to the antibody-coated wells. **Incubate for 10 minutes.**



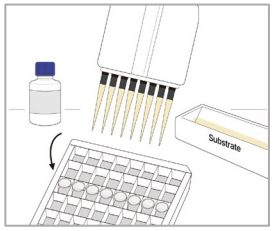
3. Carefully empty the wells and wash **5 times** with diluted wash buffer.



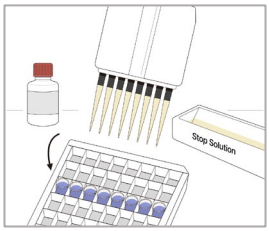
4. Tap the microwells on towels to remove all residual buffer.



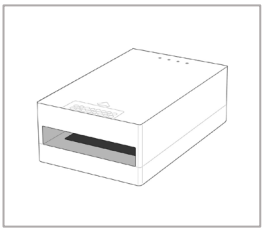
5. Add the enzyme-**conjugate solution** to the antibody-coated wells. **Incubate for 10 min. Wash as in step 3 and 4.**



6. Pipette the **substrate solution** to each microwell. **Incubate for 10 minutes in the dark.**



7. Stop the reaction by pipetting the **stop solution** to each microwell.



8. Read the absorbance of each well at **450 nm** (reference wavelength **630 nm**) with an ELISA reader. Calculate results according to the *Results Analysis* section.

Reagent and sample preparation

Buffer preparation

Wash buffer:
Dilute the concentrated wash buffer (20X) 1:20 with distilled water as described here: transfer the content of the wash buffer to 1 L screw-cap bottle and fill it up with distilled water up to the 1000 mL mark. Shake it and store it refrigerated 2-8°C (35-46°F). Label as diluted wash buffer. For each eight-well strip, approx. 50 mL of diluted wash buffer are needed.
Note: Before running the assay, allow the diluted wash buffer to equilibrate at room temperature.
Note: Diluted wash buffer is stable for four weeks if stored at 2- 8°C (35-46°F).
Note: If crystals form in any of the buffers during cold storage, the concentrate should be warmed up to 37°C (98°F) until they dissolve.

Sample preparation

Solid or liquid samples (foods):

1. Obtain a representative sample of the specimen you want to analyze and homogenize a minimum of 5 g in a mortar or blender. Weigh out **1 g or mL** of homogenized sample into a 50 mL clean centrifuge tube.
2. Add to the 50 mL centrifuge tube one capsule of extraction **additive 1** and one capsule of extraction **additive 2**.
3. Heat to 60°C (140°F) the required amount of distilled or deionized water (approx. 20 mL per sample) in a boiler or water bath.
4. Add **20 mL**(or **19 mL** if a liquid sample was used) **of hot water** to the 50 mL centrifuge tubes containing the sample and the extraction additive 1 and 2. Close the tube and **shake** vigorously for about **15 seconds** to allow capsules and sample to dissolve.
5. Take an aliquot of **1.5 mL or 2 mL** from the 50 mL centrifuge tube and transfer it to a micro-centrifuge vial. Centrifuge it at **≥ 8000 g for 5 minutes** and use the supernatant for testing.

   Note: If a centrifuge is not available, filter the extract with filter paper and then collect the filtrate.

   Note: If dilutions are required, dilution buffer can be prepared by adding 1 capsule of additive 1 and 1 of additive 2 to 20 mL of hot non-boiling water (60°C) and shaking vigorously for 15 seconds.
6. Leave the sample extract to cool down to room temperature before carrying out the ELISA. Samples are ready for testing. Please read the *ELISA procedure* section and follow carefully the protocol.

Swab samples:

1. Moisten a swab with water and rub on a selected surface while rotating the swab using horizontal movements first, then vertical.
2. Break off or cut the swab and transfer it to a 10 mL centrifuge tube.
3. For the extraction, heat to 60°C (140°F) the required amount of distilled or deionized water (approx. 20 mL per sample) in a boiler or water bath.
4. Add the hot water to a 50 mL clean centrifuge tube together with one capsule of extraction **additive 1** and one capsule of extraction **additive 2**. Close the tube and **shake** vigorously for about **15 seconds** to allow capsules to dissolve.
5. Add 2 mL of the so prepared extraction buffer to a 10 mL centrifuge tube containing the swab. Close the tube and **shake** vigorously for about **15 seconds**.
6. Transfer the liquid to a **2 mL** micro-centrifuge vial. Centrifuge it at **≥ 8000 g for 5 minutes** and use the supernatant for testing.

Note: If a centrifuge is not available, filter the extract with filter paper and then collect the filtrate.
Note: If dilutions are required, the buffer prepared in step 3 can be used as dilution buffer.

7. Leave the sample extract to cool down to room temperature before carrying out the ELISA. Samples are ready for testing. Please read the *ELISA procedure* section and follow carefully the protocol.

Rinse water samples:

1. Prepare the extraction buffer as described in the section *Solid samples* (step 2 to 5).
2. Use this buffer to dilute the rinse water sample (pH 4-9) 1:2 as described here: dispense 0.5 mL of rinse water into a vial, add 0.5 mL of buffer and shake.

Note: If the results of a sample are out of the range of quantitation or if you expect your sample to be highly contaminated, further dilution with the extraction buffer is necessary. The additional dilution factor (F) must be considered when calculating the final concentration. For more details, see section *Results Analysis*. Some samples could give negative results, but still contain allergen below the limit of detection of the test kit. The food matrix of certain foods and high degree of processing can influence the results of the test.

➲ **Did you know?** Sample extracts can be stored at 2–8°C (35-46°F) for up to 24 hours before running the assay. If longer storage is required, extracts can be stored at -20°C (-4°F) up to 2 weeks. If you follow any of these steps, do not forget to allow the sample extracts to return to room temperature and vortex before proceeding to the assay.

➲ **Did you know?** The AgraQuant® Plus Egg ELISA test kit uses its own extraction buffer. Please, do not use the same sample extract with any other allergen test kits. AgraQuant® Plus extractions cannot be used with AgraQuant® ELISA test kits.

Sample specifications

Cross-reactivity: No cross-reactivity was detected with relevant pure food commodities.

Technical support

Not sure if the test works with your specific samples or matrices? Let our longstanding experience in food allergen testing work for you. Contact our technical sales representative in your region to know more.

ELISA procedure

Before starting

Procedural guidelines:

- Make sure you have everything you need ready before starting the assay.
- All reagents and kit components must be equilibrated to room temperature, i.e. 20-25°C (68-77°F), before use.
- It is good laboratory practice to run standards and sample extracts in duplicates.
- Run a standard curve with each assay.
- Adhere to the incubation times stated in the procedure. Use of incubation times other than those specified may give inaccurate results.
- The wash procedure is critical and therefore must be performed accurately.
- Use a maximum of 3 eight-well strips in one experiment to guarantee comparable incubation times.

Precautions:

- Do not mix or interchange reagent lots from different kits lots.
- Due to the high risk of cross-contamination, all used instruments must be cleaned thoroughly before sample preparation. Adhere to the instructions for test procedures.
- Cover or cap all reagents when not in use.
- The substrate is light sensitive. Avoid direct exposure to light.
- The stop solution contains acid (1 mol/L sulfuric acid). Avoid contact with skin or eyes. If exposed, flush with water.
- Wear heat protective gloves and safety glasses when using the kit.
- Dispose of all materials and containers properly after use.

Assay protocol

1. Place an appropriate number of **white-colored wells** (transfer) and **antibody-coated wells** in the microwell strips frame. Once the desired number of strips are removed from the foil pouch, reseal the bag and store it at 2-8°C (35-46°F) to preserve it.
2. Add **150 µl of each ready-to-use standard and prepared sample** to the white-colored wells. Use a fresh pipette tip for each standard and sample.
3. Transfer **100 µl of each ready-to-use standard and prepared sample** from the white-colored well to the corresponding antibody-coated microwells with a multichannel pipette and incubate at room temperature 20-25°C (68-77°F) for **10 minutes**.
   Note: Do not attempt to mix the content of the microwells by shaking the plate as this may cause well-to-well contamination.
4. **Wash step:** Empty the content of the microwell strips into a waste container and remove any residual liquid by gently tapping the plate on paper. Pipette 300 µL of wash buffer, and then discard it again. 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 the microwell strips on towels to remove all of the residual buffer after the fifth wash.
   Note: Never insert absorbent paper directly into the wells.
6. Measure the required amount of enzyme-conjugate solution from the green-capped bottle (~120 µL/well or 1 mL/strip) and place in a separate container (e.g. reagent boat). Dispense **100 µL of enzyme-conjugate solution** into each well with a multichannel pipette. Incubate at room temperature 20-25°C (68-77°F) for **10 minutes.**

Note: Do not attempt to mix the content of the microwells by shaking the plate as this may cause well-to-well contamination.

7. **Wash step:** Perform the washing step as described above at point 4.
8. Lay several layers of absorbent paper towels on a flat surface and tap the microwell strips on towels to remove all of the residual buffer after the fifth wash.
   Note: Never insert absorbent paper directly into the wells.
9. Measure the required amount of substrate solution from the blue-capped bottle (~120 µL/well or 1 mL/strip) and dispense into a separate container (e.g. reagent boat). Pipette **100 µL of the substrate solution** into each microwell with a multichannel pipette. Incubate at room temperature 20-25°C (68-77°F) for **10 minutes** and allow the reaction to develop **in the dark** (e.g. cover completely, or carefully place in a cupboard or drawer).
10. 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). Stop the reaction by pipetting **100 µL of stop solution** into each microwell by using a multichannel pipette. The color will change from blue to yellow.
11. Read the absorbance of each wells after the addition of the stop solution at 450 nm (reference wavelength 630 nm) with a microwell reader.
    Note: Carefully remove any air bubbles prior to reading the absorbance as they may affect the result.
    Note: Do not return unused reagents to their original bottles. Carefully note which rows/strips contain standards or samples during the assay.

Results analysis

Solid/liquid samples (foods):

The content of egg in your samples can be now determined. The ready-to-use standards at known concentrations allow for the determination of the concentration of the unknown samples. The evaluation should be performed using a **four-parameter logistic curve (4PL).** Alternatively, results can be easily calculated using the **Romer Labs® spreadsheet** that is provided free of charge upon request. With the Romer Labs® spreadsheet you only need to insert the obtained OD values and the allergen amounts in your samples are calculated automatically using the point-to-point curve fitting.
Note: An OD value of less than 1.1 absorbance units for the highest standard may indicate the deterioration of reagents. If the substrate solution has a blue color before being added to the wells, might be that the reagent is no longer suitable for use.
Note: If the percent coefficient of variation (%CV) of the duplicate readings of the standards or of the samples exceeds 20%, the result of your test might be inaccurate. Repeat the assay.

When working according to the *sample preparation* section described in this package insert, a dilution factor of 20 is applied during sample extraction. This dilution factor of 20 is already taken into account, thus the allergen concentration can be read directly from the standard curve obtained. If the amount of allergen contained in a sample exceeds the value of the highest standard (whole egg powder >25 ppm), the sample extract should be further diluted by using the extraction buffer prepared as explained in the section *sample preparation* and then re-analyzed by performing a new assay. This step places your sample within the range of quantification of the AgraQuant® Plus Egg ELISA test kit range (1 - 25 ppm) so that you can obtain accurate results.

The dilution factor (F) of this additional dilution step must be applied when the result is calculated as follows:

$$\textbf{whole egg powder [mg/kg or mg/L]} = \frac{\textbf{value from the standard curve x dilution factor (F)}}{\textbf{sample weight [g or mL]}}$$

Swab samples:

When running a swab sample, use the concentration you read off the curve to perform the calculation as below:

whole egg powder [µg/swab] = value from the standard curve x dilution factor (F) x 0.1

Rinse water samples:

When running a rinse water sample, use the concentration you read off the curve to perform the calculation as below:

whole egg powder [µg/L] = value from the standard curve x dilution factor (F) x 100

Visit **www.romerlabs.com** to find worldwide contact information.
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