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Product Manual

AgraQuant® Gluten G12® ELISA test kit
Article number 10001994

Intended use
The AgraQuant® Gluten G12® ELISA test kit is an immunoassay designed for the quantitative analysis of gluten in food samples. Samples can vary from raw to processed foods, from environmental swabs to rinse water. This product is intended for laboratory use.

Minimum performance characteristics

Limit of detection (LOD): 2 ppm (2 mg/kg) gluten

Limit of quantification (LOQ): 4 ppm (4 mg/kg) gluten

Range of quantification: 4 – 200 ppm (4 - 200 mg/kg) gluten

Plate format: 96 wells

Assay time: sample preparation – 120 minutes (approx.)
total incubation time – 60 minutes

About Gluten and the G12® antibody
Gluten is a group of storage proteins from cereals (in particular wheat, rye, barley and related species), that consists of prolamins (in wheat: gliadin) and glutelins (in wheat: glutenins). Coeliac disease is a complex enteropathy caused by the exposure of predisposed individuals to gluten. It is a life-long disease; persons suffering from it experience severe symptoms when consuming even very small amounts of gluten. The onset of the disease has been linked to the presence of one particular 33-mer peptide from gliadin. The G12® antibody detects this immunogenic peptide, ensuring that the most relevant harmful agent from gluten is absent from the samples tested. Gluten is widely used as a binder in food products due to its physicochemical characteristics. Moreover, cross-contamination of food products and production lines with gluten is often observed. The detection of gluten in products and production lines is therefore of utmost importance. To comply with Codex Standard 118-1979, products labeled “gluten-free” must have gluten levels below 20 mg/kg, while those specially processed to reduce gluten content must keep their levels between 20 and 100 mg/kg.

The AgraQuant® Gluten G12® ELISA test kit is approved as:
- AACC International Method 38-52.01
- AOAC Official Method of Analysis (OMA) 2014.03

Product information

About the ELISA test kit

The AgraQuant® Gluten G12® test kit is an enzyme-linked immunosorbent assay (ELISA) sandwich used for the quantification of gluten in food samples. This product is a very sensitive detection system and utilizes highly purified monoclonal antibodies raised against the 33-mer peptide for the quantification of gluten in a variety of food products. With AgraQuant® Gluten G12®, raw materials and finished food samples can be tested for the presence of gluten. AgraQuant® Gluten G12® can also be used to validate cleanings and to test for the presence of gluten via rinse waters or environmental swab.

Storage information

Upon receipt, immediately transfer the AgraQuant® Gluten G12® 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® Gluten G12® ELISA test kit contains the following items:

- 96 antibody-coated microwells (12 eight-well strips) in a microwell holder sealed in a foil pouch
- 5 vials of 1.2 mL each of gluten G12® standard (0, 4, 20, 80 and 200 ppm)
- 1 green-capped bottle of 12 mL of enzyme-conjugate solution (detection antibody)
- 1 blue-capped bottle of 15 mL of substrate solution
- 1 red-capped bottle of 15 mL of stop solution
- 1 bottle of 110 mL of ready-to-use extraction buffer (bottle A)
- 1 bottle of 20 mL of 5X concentrated dilution buffer (bottle B)
- 1 bottle of 60 mL of 10X concentrated wash buffer (bottle C)
- 1 sachet of 10 g of fish gelatin

Materials required but not included

Extraction Procedure:

- Blender, or mortar and pestle, or homogenizer
- Analytical balance
- Graduated cylinder, 100 mL
- Distilled or deionized water for diluting concentrated buffers
- 80/20 (v/v) Ethanol/water
- Container with a minimum capacity of 10 mL
- Centrifuge, micro-centrifuge (or filter and funnel) and centrifuge tubes
- Water bath (50°C/122°F)
- Laboratory shaker

Assay Procedure:

- Calibrated 8-channel and single-channel pipettes with 100 µL disposable plastic tips
- Timer
- Plate washer or wash bottle
- Distilled or deionized water
- Absorbent paper towels
- 3 reagent boats for use as reagent containers for an 8-channel pipette
- Microwell reader with a 450 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® ELISA test kits are based on solid phase sandwich ELISA technology. The kit comes with monoclonal antibodies raised against the immunotoxic 33-mer from gluten, pre-coated onto each well. During the first incubation, both samples and standards are added into the wells to allow any gluten present in the sample to bind to the immobilized antibodies.
After washing, the enzyme-conjugated solution (detection antibody) is added into the wells and binds to the immobilized molecules captured during the first incubation, forming a "sandwich". After a second washing step to remove any excess of detection antibodies, the substrate solution is added, which results in color development. The intensity of the color developed is proportional to the concentration of gluten 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. 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.

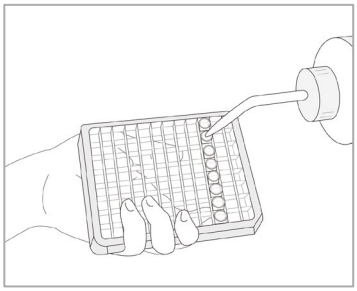
Protocol at a glance

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

1

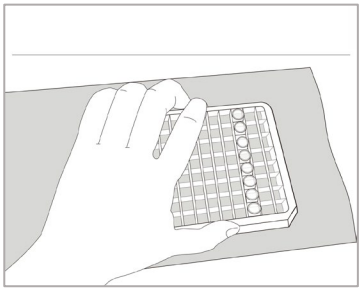
Dispense **samples** and **standards** into the antibody-coated wells. **Incubate for 20 min**.

2



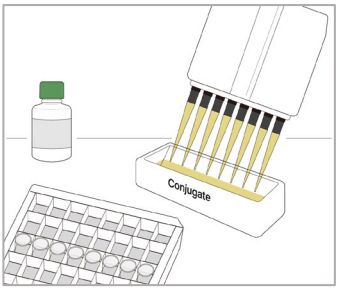
Carefully empty the mirowells and wash **5 times** with diluted wash buffer.

3



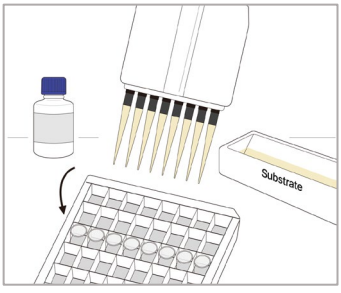
Tap the microwell strips onto absorbent paper towels to remove any residual buffer.

4



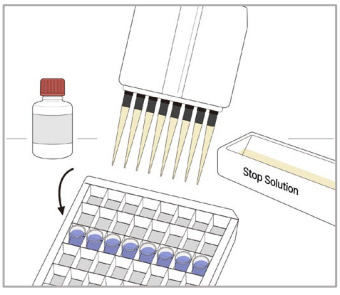
Pipette the **enzyme-conjugate solution** into the microwells.
Incubate for 20 min.
Wash as in steps 2 and 3.

5



Pipette the **substrate solution** into each microwell.
Incubate for 20 minutes in the dark.

6



Stop the reaction by pipetting the **stop solution** into each microwell.

7. Read the absorbance of each well at 450 nm with an ELISA reader. Calculate results according to the *Results Analysis* section.

Reagent and sample preparation

Buffer preparation

Ethanol 80%:
To be used in combination with the extraction buffer. Prepare a 80/20 (v/v) ethanol/water solution for the extraction of your samples: add 120 mL of ethanol to 30 mL of distilled water to obtain a final volume of 150 mL, and shake well.

Dilution buffer:
Calculate the amount of dilution buffer needed according to the number of samples and prepare a 1:5 solution from the concentrated dilution buffer (5X, bottle B) with distilled water (e.g., add 20 ml of concentrated dilution buffer to 80 mL distilled water for obtaining a final volume of 100 ml dilution buffer).
Note: The diluted dilution buffer is stable for up to one week if stored at 2-8°C (35-46°F).

Wash buffer:
Dilute the concentrated wash buffer (10X, bottle C) 1:10 with distilled water (e.g. add 10 mL of concentrated wash buffer to 90 mL distilled water to obtain a final volume of 100 mL wash buffer).
Note: If crystals form in the buffers during cold storage, the concentrates should be warmed up to 37°C (98°F) until they dissolve. Diluted wash buffer is stable for four weeks if stored at 2- 8°C (35-46°F).

Sample preparation

Solid samples:

1. Obtain a representative sample of the specimen you want to analyze and homogenize a minimum of 5 g in a mortar or blender, to a powder as fine as possible.
2. Weigh out 0.25 g of homogenate into a test tube with a minimum capacity of 10 mL and add **2.5 mL of extraction buffer (bottle A)**. Work under a fume/chemical hood. Vortex or shake until the sample is completely dispersed in the extraction buffer.
   Note: When testing chocolate-containing samples add 0.25 g of the provided fish gelatin before adding the extraction buffer.
3. Incubate the extract in a pre-warmed water bath at 50°C/122°F for 40 minutes and vortex it at regular intervals.
4. Allow the sample extract to cool down (20-25°C/67-77°F) and add 7.5 mL of **ethanol 80%.** Vortex it.
5. Shake the sample extract for 60 minutes at room temperature (20-25°C/67-77°F) in a laboratory shaker. Check the sample after 30 minutes and vortex it if clumps have formed, to disperse them. Then, place it back onto the shaker to complete the extraction procedure for 30 more minutes.
6. Centrifuge samples for 10 minutes at 2000 g to obtain a clear supernatant (depending on the sample matrix, the supernatant can also appear as a clear aqueous layer between the particulate and a fat layer). Collect the supernatant and transfer it into a clean vial.
7. Dilute the supernatant 1:10 with dilution buffer prepared as stated on the “Buffer preparation” section (e.g., 100 µL of sample extract with 900 µl dilution buffer). Vortex the solution. This dilution factor (10) is already taken into account in the final calculation.

Liquid samples:

1. Obtain a representative sample of the specimen you want to analyze.
2. Take 0.25 mL of liquid sample into a test tube with minimum of 10 mL capacity and add **2.25 mL extraction buffer (bottle A)**. Mix well by shaking or with a Vortex.
3. Continue with point 3. of the procedure described in the section Solid samples of the previous page.

Swab samples

If you are using our AgraQuant® Allergen Swabbing Kit (10002205) for collecting environmental swab samples, you can analyze the gluten content of the swab with the AgraQuant® Allergen ELISA test kit. Please carefully follow the package insert of the AgraQuant® Allergen Swabbing Kit. The obtained sample is ready to use and does not need to be further extracted with the dilution buffer from the AgraQuant® Gluten G12® ELISA test kit. Pipette 100 µl of your extracted swab sample directly into the wells. Different ranges of quantification apply for the swabs. For more details, please refer to the package insert of AgraQuant® Allergen Swabbing Kit.

Note: The AgraQuant® Gluten G12® ELISA test kit uses its own extraction and dilution buffers. Please, do not use the same sample extract with any other allergen test kits.

General recommendations

If you expect your sample to be highly contaminated, further dilution with the dilution buffer is necessary. The additional dilution factor must be considered when calculating the final concentration. For more details, see section Results Analysis.

Some samples could give negative results, but still contain gluten 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.

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., 18-30°C (64-86°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.
- It is strongly recommended that the assay be performed with an 8-channel pipette.
- The wash procedure is critical and therefore must be performed accurately.
- Do not run more than 6 eight-well strips in one experiment when using an 8-channel pipette.

Precautions:

- Do not mix or interchange reagent 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.
- Close or cover all reagents when not in use.
- The stop solution contains acid. Avoid contact with skin or eyes. If exposed, flush with water.
- Wear protective gloves and safety glasses when using the kit.
- Dispose of all materials and containers properly after use.

Assay protocol

Optional Transfer well method:

1. Place an adequate number of **transfer wells** in a microwell strip holder and then add **150 µl of each ready-to-use standard and prepared sample** into them. Use a fresh pipette tip for each standard and sample. Make sure to empty the pipette tip completely each time.
2. Place an appropriate number of **antibody-coated microwells** in a microwell strip holder and, using an 8-channel pipette, transfer **100 µl of each ready-to-use standard and prepared sample** from the transfer wells to the corresponding antibody-coated microwells.

Continue with step 3 of the standard assay protocol.

Standard assay protocol:

1. Place an appropriate number of **antibody-coated microwells** in a microwell strip holder. 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. Using a single channel pipette, add **100 µL of both ready-to-use standards and prepared samples** into the appropriate well. Use a fresh pipette tip for each standard or sample. Make sure to empty the pipette tip completely.

3. Incubate at room temperature for **20 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. **Washing:** Empty the content of the microwell strips into a waste container. Wash by filling each microwell with wash buffer (prepared as stated in the "Buffer preparation" section), 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. After the fifth wash, lay several layers of absorbent paper towels on a flat surface and tap the microwell strips on top of them to remove all the residual buffer.
   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 an 8-channel pipette.
7. Incubate at room temperature for 20 minutes.
8. **Washing:** Wash the plate 5 times as described above at steps 4 and 5.
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 an 8-channel pipette.
10. Incubate at room temperature for **20 minutes** and allow the reaction to develop **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). Stop the reaction by pipetting **100 µL of stop solution** into each microwell by using an 8-channel pipette. The color will change from blue to yellow.
12. Read the absorbance of each well at 450 nm with a microwell reader. Carefully remove any air bubbles prior to reading the absorbance as they may affect the accuracy of the result.
    Note: Do not return unused reagents to their original bottles. Carefully track which wells contain standards and which samples during the assay.

Results analysis

The content of gluten in your samples can now be determined. The ready-to-use standards at known concentrations allow for the determination of the concentration of the unknown samples.
Calculate the mean value of the optical density (OD) of the duplicates for each standard. Build a dose-response curve using the five standards by plotting the mean OD obtained for each of the five standard (y-axis) against its concentration in ppm (x-axis). Determine the corresponding concentration of gluten in ppm by interpolation from the standard curve using a point-to-point calculation. If you are using a software-based quantification system, the four parameter logistic curve (4PL) may be used.
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 gluten amounts in your samples are calculated automatically using the point-to-point curve fitting.
When working according to the sample preparation section described in this package insert, a dilution factor of 400 is applied during sample extraction. This dilution factor of 400 is already taken into account, thus the gluten concentration can be read directly from the standard curve obtained.

If the amount of gluten contained in a sample exceeds the value of the highest standard (gluten > 200 ppm), the sample extract should be further diluted by using dilution buffer and then re-analyzed by performing a new assay. The dilution should be done such that the diluted sample results are within the AgraQuant® Gluten G12® ELISA test kit quantification range (4 - 200 ppm). The dilution factor of this additional dilution step must be applied when the result is calculated.
Notes: An OD value of less than 1.1 absorbance units for the 200 ppm standard may indicate the deterioration of reagents. 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. It is recommended to repeat the assay.

Swab samples: When running a swab sample, you can calculate the concentration of your sample, by using the concentration you read off the curve, as in the following example:

Value read off curve: **5 ppm**
Multiply this value by 1000 to convert the units to ng/ml: 5 ppm X 1000 = **5000 ng/ml**
Since there was no dilution performed during preparation nor extraction, divide this value by the dilution factor considered in the curve (400): 5000xng/ml ÷ 400 = **12.5 ng/ml.**

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