AgraQuant® Gluten G12 Assay (4 - 200 ppm)

Order #: COKAL0200

Intended Use

The AgraQuant® Gluten G12 Assay is a sandwich enzyme-linked immunosorbent assay (ELISA) that determines a quantitative level for the presence of specific immunotoxic gluten fragments in a variety of foods.

The AgraQuant® Gluten G12 Assay represents the next generation of highly sensitive detection systems that are designed for the detection of toxic gluten fragments that are responsible for triggering the immune reactions in coeliac sufferers, thereby determining the relative safety of foods for coeliacs.

Approvals

The AgraQuant® Gluten G12 Assay is approved:

- as ***AACC International Method 38-52.01***
- as well as ***AOAC Official Method of Analysis (OMA) 2014.03***

Gluten G12

Gluten is the main group of proteins in grains and consists of prolamins (in wheat: gliadin) and glutelins (in wheat: glutenins) occurring in the same ratio. Due to its physicochemical characteristics, gluten is used in food products to contribute texture and form. Coeliac disease is an autoimmune disorder of the small intestine. It is caused by a reaction to a toxic 33-mer peptide and the only effective treatment is a lifelong gluten-free diet. Due to the Codex Standard 118-1979 and labelling regulations (e.g. EC Directive 41/2009), "gluten-free" products must comply with gluten levels (including prolamin fractions from rye, barley and oats) below 20 mg/kg and "foods specially processed to reduce gluten content" must comply with levels between 20 and 100 mg/kg, to be labelled "very low gluten" products.

Assay Principles

The AgraQuant® Gluten G12 assay is a sandwich enzyme-linked immunosorbent assay (ELISA). Gluten is extracted from samples using extraction buffer. Monoclonal antibodies directed against the toxic fraction of gluten (G12) are pre-coated on the surface of a microwell. The extracted sample or standards are applied to the wells and the gluten binds to the antibodies. After a washing step, an enzyme-conjugated monoclonal antibody specific to the Gluten G12 protein is applied to the well and incubated. After a second washing step, an enzyme substrate is added and blue colour develops. The intensity of

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the colour is directly proportional to the concentration of Gluten G12 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 (OD_{450}). The optical densities of the samples are compared to the OD's of the standards and an interpolated 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 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. The Extraction Solution contains chemicals which are harmful to health. Sample extraction should take place under a chemical hood and contact with the skin avoided.
6. Wear protective gloves and safety glasses when using the kit.
7. Dispose of all materials, containers and devices appropriately after use.

Materials Supplied With Kit

- 96 antibody coated microwells (12 eight-well strips) in a microwell holder (sealed in a foil pouch)
- 5 vials of 1.2 mL of each Gluten G12 standard (0, 4, 20, 80 and 200 ppm)
- 1 bottle of 12 mL of Conjugate (green-capped bottle)
- 1 bottle of 15mL of Substrate solution (blue-capped bottle)
- 1 bottle of 15 mL of Stop solution (red-capped bottle)
- 1 bottle of 20 ml of 5x concentrated Diluent buffer
- 1 bottle of 60 mL of 10x concentrated Wash buffer
- 1 bottle of 105 ml of ready to use Extraction solution.
- 1 sachet of 10 g of Fish Gelatin

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
- 80/20 (v/v) Ethanol/water
- Container with a minimum 10mL capacity
- Flask Shaker (Stuart SF1 or equivalent)
- Centrifuge, Microcentrifuge or Filter and Funnel
- Centrifuge tubes
- Water bath (50°C)

Assay Procedure

- *8-channel and single channel pipettors capable of pipetting 100 µL 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

Ethanol 80%

For use with the Extraction solution. Add 120 ml ethanol to 30 ml distilled water and shake well

Diluent Buffer

Dilute Diluent buffer concentrate 1:5 with distilled water (e.g. add 20 mL of concentrated Diluent buffer to 80 mL distilled water). Store at 4°C. The diluted Diluent 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 concentrate 1:10 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 homogenise a minimum of 5 g in a mortar or blender as fine as possible.
2. Weigh out 0.25 g of homogenized sample into a vial with a minimum 10 mL capacity, which can be tightly sealed. (For chocolate containing samples additionally add 0.25 g of powdered fish gelatin.) Add 2.5 mL of the extraction solution (under a fume/chemical hood), close vials and mix vigorously on a vortex. Visually check for clumps, keep vortexing until the samples are well-dispersed in the extraction solution.
3. Incubate extracts at 50°C for 40 minutes. Mix well, at regular intervals (at least twice) during the incubation using a laboratory vortex.
4. Allow the extracts to cool and add 7.5 ml of 80% Ethanol. Mix well using a laboratory vortex
5. Shake for a total of 60 min at room temperature (20-25°C/68-77°F) with a laboratory rotator. (After about 30 min in the rotator, check the vials visually if all sample

material has suspended in the liquid – if clumps have formed vortex and let the vials rotate for the 2nd 30 minutes to complete the extraction procedure)

6. Centrifuge samples for 10 min at 2000 x g to obtain a clear aqueous layer between the particulate sediment and supernatant. Note in some cases a thin fatty layer creaming on top of the supernatant. Collect the aqueous supernatant (extract) and transfer into new a vial.
7. Dilute supernatant 1:10 with pre-diluted sample dilution buffer (e.g. 100 µL particle-free sample solution in 900 µL dilution buffer). Mix well using a laboratory vortex.

Note: This dilution factor of 10 is already considered in the calculation. If the results of a sample are out of the quantitation range further dilution with the sample dilution buffer is required. The additional dilution has to 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 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). If using only single channel pipettes, it is recommended that no more than a total of 16 samples and standards be run in one experiment (8 when standards and samples are added in duplicate e.g. 2 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 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 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 on the next page

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 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.**
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 (~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.**
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 (~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 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.

Interpretation of the Results

Using either the unmodified OD values or the OD values expressed as a percentage of the OD of the 200 ppm standard, construct a dose-response curve using the five standards. Since the amount of Gluten 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 200 ppm standard may indicate deterioration of reagents.

If a sample contains Gluten levels higher than the highest standard (>200 ppm), the sample extract should be further diluted in dilution buffer such that the diluted sample results are in the range of 4 - 200 ppm and reanalysed to obtain accurate results. The dilution factor must be included when the final result is calculated.

Content of swab samples

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

Value for swab sample read off curve = 5 ppm
To convert into ng/ml =5 X 1000 = 5000 ng/ml
As no extraction step was used (1/400 extraction) = 5000/400 = 12.5 ng/ml

Performance Characteristics

Limit of detection:	2 ppm Gluten
Limit of quantitation:	4 ppm Gluten
Range of quantitation:	4– 200 ppm (For quantitation of samples above 200 ppm, samples should be diluted such that the diluted sample results are in the range of 4 - 200 ppm).
No Cross Reactivity to:	Pecan, Walnut, Almond, Cashew, Macadamia, Peanut, Hazelnut, Pine Nuts, Pistachio, Linseed, Poppy Seed, Sesame, Mustard, Soya, Millet, Buckwheat, Quinoa, Fennel, Chickpea, Coriander, Black Eyed Beans, Puy Lentils, Cardamom .

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