AgraQuant® Ovalbumin Assay (25 – 500 ppb)

Order #: COKAL2948

Intended Use

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

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

Ovalbumin

Hen's egg (Gallus gallus) is very rich of proteins and represents an important food source for humans. While proteins of egg yolk only have minor allergenicity, many proteins of egg white are known to be allergenic. In addition to lysozyme, ovomucoid ovotransferrin and livetin, ovalbumin represents the main fraction of the egg white allergens. Amongst others egg powder as well as pure ovalbumin is often used as fining reagent for wine. For allergic persons the consumption of ovalbumin represents a critical problem. Already very low amounts of the allergen can cause allergic reactions, which may lead to anaphylactic shock in severe cases. Because of this, ovalbumin allergic persons must strictly avoid the consumption of ovalbumin containing food. Non-declared addition of ovalbumin in food is hazardous for allergic people. Cross-contamination, mostly in consequence of the production process, is often noticed. Since July 1, 2012 the European Union requests allergen labeling for wine if milk or egg proteins are used during the production and are still present at a detection level of 0.25 mg/L or greater. Thus for the detection of ovalbumin residues, sensitive assay systems are required.

Assay Principles

The AgraQuant® Ovalbumin Assay is a sandwich enzyme-linked immunosorbent assay (ELISA). Ovalbumin proteins are extracted from a sample using an extraction buffer. Antibodies directed against Ovalbumin proteins are pre-coated on the surface of a microwell. The extracted sample or standards are applied to the wells and the Ovalbumin proteins bind to the antibodies. After a washing step, an enzyme-conjugated antibody specific to Ovalbumin 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 Ovalbumin 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. 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 0.75 mL of each Ovalbumin Standard (0, 25, 100, 250 and 500 ppb)
- 1 bottle of 6 mL of Conjugate (green-capped bottle)
- 1 bottle of 6 mL of Substrate solution (blue-capped bottle)
- 1 bottle of 6 mL of Stop solution (red-capped bottle)
- 1 bottle of 120 mL of 10x concentrated Extraction solution
- 1 bottle of 30 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.

Ovalbumin Standards
Ovalbumin Standards (0, 25, 100, 250, 500 ppb of Ovalbumin) 5 vials with 1.0mL, ready to use

Procedure

Sample Preparation / Extraction

Wine Samples
1. 1 ml of the wine sample is diluted in 19 mL of pre-diluted extraction buffer.
2. 100 µL of the wine solution are applied per well. If the results of a sample are out of the measuring range, further dilution with the pre-diluted extraction and sample dilution buffer is necessary. The additional dilution has to be considered when calculating the concentration

Other Samples
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® Ovalbumin Assay Kit for the determination of Ovalbumin 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). 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 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 500 ppb standard, construct a dose-response curve using the five standards. Since the amount of Ovalbumin 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 0.7 absorbance units for 500 ppb standard may indicate deterioration of reagents.

If a sample contains Ovalbumin levels higher than the highest standard (>500 ppb), the sample extract should be further diluted in extraction buffer such that the diluted sample results are in the range of 25 – 500 ppb and reanalysed to obtain accurate results. The dilution factor must be included when the final result is calculated.

Ovalbumin content of swab samples

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

Value for swab sample read off curve = 50 ppb
To convert into ng/ml =50 ng/ml
As no extraction step was used (1/20 extraction) = 50/20 = 2.5 ng/ml

Performance Characteristics

Limit of detection: 4 ppb Ovalbumin

Limit of quantitation: 25 ppb Ovalbumin.

Range of quantitation: 25 – 500 ppb (For quantitation of samples above 500 ppb, samples should be diluted such that the diluted sample results are in the range of 25 - 500 ppb).

Cross Reactivity :

Lysozyme	< 0.02%
Ovomucoid	< 0.02%
Conalbumin	< 0.2%
Non Fat Dry Milk	0%
Fish	0%

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