AccuDiag™
Egg White
ELISA

Cat# 5138-8

Test	Egg White
Method	Enzyme Linked Immunosorbent Assay
Principle	Sandwich Complex
Sample	1 g
Total Time	~ 60 min.
Shelf Life	12-18 Months from the manufacturing date
Sensitivity	0.05 ppm

INTENDED USE

Diagnostic Automation, Inc. 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 ovalbumin, ovotransferrin, lysozyme and livetin, ovomucoid represents the most important allergen. Unlike the other allergens ovomucoid is heat stable and can resist common production processes like baking. For allergic persons the consumption of egg white 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, egg allergic persons must strictly avoid the consumption of eggs or egg containing food. Non-declared addition of egg in food is hazardous for allergic people. Cross contamination, mostly in consequence of the production process is often noticed. The chocolate production process is a representative example. For the detection of egg white protein residues, sensitive detection systems are required.

SUMMARY AND EXPLANATION

The Diagnostic Automation, Inc. Egg White ELISA represents a highly sensitive detection system for egg white protein based on ovomucoid and is particularly capable of the quantification of egg white residues in pasta, bakery products, sausage and chocolate.

TEST PRINCIPLE

The DAI **Egg White** quantitative test is based on the principle of the enzyme linked immunosorbent assay. An antibody directed against ovomucoid is bound on the surface of a microtiter plate. Egg white (ovomucoid) containing samples or standards are given into the wells of the microtiter plate. After 20 minutes incubation at room temperature, the wells are washed with diluted washing solution to remove unbound material. A peroxidase conjugated second antibody directed against ovomucoid is given into the wells and after 20 minutes of incubation the plate is washed again. A substrate solution is added and incubated for 20 minutes, resulting in the development of a blue colour. The color development is inhibited by the addition of a stop solution, and the color turns yellow. The yellow color is measured photometrically at 450 nm. The concentration of ovomucoid and with this the concentration of egg white is directly proportional to the color intensity of the test sample.

MATERIALS AND COMPONENTS

Materials provided with the test kits

The kit contains reagents for 96 determinations. They have to be stored at 2-8°C. Expiry data are found on the labels of the bottles and the outer package.

1. **Microtiter plate** consisting of 12 strips with 8 breakable wells each, coated with anti-ovomu-coid antibodies.
2. **Egg white protein standards** (0; 0.4; 1; 4; 10 ppm of egg white protein): 5 vials with 1.0 mL each dyed red, ready-to-use.
3. **Conjugate (anti-ovomucoid-peroxidase):** 15 mL, dyed red, ready-to-use.
4. **Substrate Solution (TMB):** 15 mL, ready-to-use.
5. **Stop Solution (0.5 M H_2SO_4):** 15 mL, ready-to-use.
6. **Extraction and sample dilution buffer (Tris):** 2 x 120 mL as 10x concentrate, dyed red. Dilute 1+9 with distilled water. Stored at 4°C the diluted buffer is stable for at least one week. If during the cold storage crystals precipitate, the concentrate should be warmed up to 37°C for 15 minutes.
7. **Washing Solution (PBS + Tween 20):** 60 mL as 10x concentrate. Dilute 1+9 with distilled water. Stored at 4°C the diluted buffer is stable for at least 4 weeks. If during the cold storage crystals precipitate, the concentrate should be warmed up to 37°C for 15 minutes.
8. Plastic bag to store unused microtiter strips.
9. Instruction Manual.

Materials required but not provided

1. 100 - 1000 µL micropipettes.
2. Volumetric flask.
3. Analytical balance.
4. Mortar, mixer.
5. Water bath.
6. Centrifuge.
7. ELISA reader (450 nm).

Reagents

1. Double distilled water.

Sample Preparation

Due to high risk of cross-contamination all applied instruments like applicator, mortar, glass vials etc. have to be **cleaned thoroughly** before and after each sample. To identify possible cross-contamination caused by previous extractions, it is strongly recommended to note the sequence of the extractions.

1. The following sample preparation should be applied for all kinds of samples: To maximize homogeneity and representativeness of the sample drawing, a minimum of 5 g sample should be pulverized finely in a mortar, impact mill etc.
2. 1 g of the homogenized mixture is suspended in 20 mL of **pre-diluted** extraction buffer. Afterwards the suspension is incubated for 15 min in a pre-heated water bath at 60°C. To ensure good homogeneity, the samples should be shaken every two minutes.
3. The samples are centrifuged for 10 minutes at 2000 g. If it is not possible to separate the supernatant from the precipitate completely, the suspension should be filtrated if necessary.
4. 100 µL of particle-free 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.

ASSAY PROCEDURE

The washing solution is supplied as 10x concentrate and has to be **diluted** 1+9 with double distilled water before use.

In any case the **ready-to-use** standards provided should be determined twofold. When samples in great quantities are determined, the standards should be pipette ted once before the samples and once after the samples. For final interpretation the arithmetic mean is used for calculation.

In consideration of GLP and quality control requirements a duplicate measurement of samples is recommended.

The procedure is according to the following scheme:

1. Prepare samples as described above.
2. Pipette 100 µL **ready-to-use** standards or prepared samples in duplicate into the appropriate wells of the microtiter plate.
3. Incubate for 20 minutes at room temperature.
4. Wash the plate three times as follows: Discard the contents of the wells (dump or aspirate). Pipette 300 µL of diluted washing solution into each well. After the third repetition empty the wells again and remove residual liquid by striking the plate against a paper towel. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbencies.
5. Pipette 100 µL of conjugate (anti-ovomucoid-peroxidase) into each well.
6. Incubate for 20 minutes at room temperature.
7. Wash the plate as outlined in 4.
8. Pipette 100 µL of substrate solution into each well.
9. Allow the reaction to develop in the dark (e.g. cupboard or drawer; the chromogen is light-sensitive) for 20 minutes at room temperature.
10. Stop enzyme reaction by adding 100 µL of stop solution (0.5 M H_2SO_4) into each well. The blue color will turn yellow upon addition.
11. After thorough mixing, measure absorbance at 450 nm (reference wavelength 620 nm), using an ELISA reader. The color is stable for 30 minutes.

RESULTS

Validating the Results

The ready-to-use standards are prepared for a direct determination of sample concentrations. The dilution of samples in the extraction process as described in the above stated sample preparation procedure is already considered. Additional dilution due to high sample concentration has to be accounted for.

1. Calculate the average optical density (OD 450 nm) for each set of reference standards or samples.
2. Construct a standard curve by plotting the mean optical density obtained for each reference standard against its concentration in ppm on semi-log graph paper with the optical density on the vertical (y) axis and the concentration on the horizontal (x) axis. Alternatively the evaluation can be carried out by software. In this case the 4-parameter method should be preferred.
3. Using the mean optical density value for each sample, determine the corresponding concentration of egg white protein in ppm from the standard curve. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed.

Typical Standard Values

1. The following table contains an example for a typical standard curve. The binding is calculated as percent of the absorption of the 10 ppm standard. These values are only an example and should not be used instead of the standard curve which has to be measured in each new test.

Egg white protein (ppm)	% binding of 10 ppm
10	**100**
4	**74**
1	**34**
0.4	**17**
0	**3**

PERFORMANCE CHARACTERISTICS

Sensitivity

The limit of detection (LOD) of the Diagnostic Automation, Inc. **Egg White test** is 0.05 ppm.
The limit of quantification (LOQ) of the Diagnostic Automation, Inc. **Egg White test** is 0.4 ppm.

Due to the variety of sample matrices and their influence on the blank, results less than the LOQ should be treated as negative.

Cross-reactivity

For the following foods no cross-reactivity could be detected:

Cow's milk	Poppy seed	Chestnut
Sheep's milk	Sesame	Macadamia nut
Wheat	Pine nut	Lecithin
Rye	Cashew nut	Peach
Oats	Peanut	Plum
Barley	Hazelnut	Apricot
Rice	Pecan	Cherry
Corn	Brazil nut	Cocoa
Buckwheat	Coconut	Beef
Soy	Walnut	Pork
Sunflower seeds	Pistachio	Sugar
Fish gelatin	Isinglass	

The following cross-reactions were determined:

Reagent	Cross-Reactivity (%)
Ovalbumin	0,25
Ovomucoid	614
Conalbumin	2,6
Lysozyme	<0,0003
Chicken meat	< 0,001

Precision

Intra-assay Precision	4 - 9%
Inter-assay Precision	3 – 7%
Inter-lot Precision	5 - 11%

Linearity

The serial dilution of spiked samples (pasta, biscuit, cookies, sausage and chocolate) resulted in a dilution linearity of 93% - 112%.

Recovery

Mean recovery was determined by spiking samples with different amounts of egg white protein:

Pasta	91%
Biscuit	83%
Cookies	85%
Sausage	98%
Dark Chocolate	82%

PRECAUTION

Full compliance of the following good laboratory practices (GLP) will determine the reliability of the results:

1. Prior to beginning the assay procedure, bring all reagents to room temperature (20-25°C).
2. All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
3. Once the assay has been started, all subsequent steps should be completed without interruption and within the recommended time limits.
4. Replace caps in all the reagents immediately after use. Do not interchange vial stoppers.
5. Use a separate disposable tip for each specimen to prevent cross-contamination.
6. All specimens and standards should be run at the same time, so that all conditions of testing are the same.
7. Do not mix components from different batches.
8. Do not use reagents after expiration date.
9. Check both precision and accuracy of the laboratory equipment used during the procedure (micropipette , ELISA reader etc.)

Health and safety instructions

1. Do not smoke or eat or drink or pipette by mouth in the laboratory.
2. Wear disposable gloves whenever handling patient specimens.
3. Avoid contact of substrate and stop solution with skin and mucosa (possible irritation, burn or toxicity hazard). In case of contact, rinse the affected zone with plenty of water.
4. Handling and disposal of chemical products must be done according to good laboratory practices (GLP).

REFERENCES

1. Yeung JM, et al. (2000) – Determination of egg proteins in food products by enzyme immuno-assay. J AOAC Int. 83(1):139-43
2. Hefle SL, et al. (2001) – Development of a sandwich enzyme-linked immunosorbent assay for the detection of egg residues in processed foodstuff. J Food Prot. 64(11):1812-6
3. Watanaba H, et al. (2005) – Study on detection of allergenic substances (egg and milk) in processed meat products and frozen foods. Shokuhin Eiseigaku Zasshi. 46(4):139-47
4. Li Y, et al. (2008) – Establishment of a highly sensitive enzyme-linked immunosorbent assay specific for ovomucoid from hen's egg white. J Agric Food Chem. 56(2):337-42
5. Bernhisel-Broadbent J, et al. (1994) – Allergenicity and antigenicity of chicken egg ovomucoid (Gal d III) compared with ovalbumin (Gal d I) in children with egg allergy and in mice. Allergy Clin Immunol. 93(6):1047-59
6. Sakai K, et al. (1998) – Appearance of ovomucoid in coagulated egg yolk passing through from soluble fraction of coagulated egg white in boiled egg. Arerugi. 47(11):1176-81
7. Lee JW, et al. (2002) – Allergenicity of hen's egg ovomucoid gamma irradiated and heated under different pH conditions. J Food Prot. 65(7): 1196-9
8. Junko H, et al. (2004) – Recognition of native and/or thermally induced denatured forms of the major food allergen, ovomucoid (…). Biosci Biotechnol Biochem. 68(12):2490-7
9. Rolland JM, et al. (2008) – Specific and sensitive enzyme-linked immunosorbent assays for analysis of allergenic food proteins (…). J Agric Food Chem. 56(2):349-54