GENERAL INFORMATION

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. Even 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. Crosscontamination, 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.

PRINCIPLE OF THE METHOD

The Egg White ELISA is an enzyme immunoassay for quantitative analysis of egg white residues in pasta, bakery products, sausage and chocolate.

Ovomucoid in the sample binds to a specific antibody immobilized on the microwell surface. In a second incubation, a peroxidase conjugated antibody directed against egg white proteins binds to surface-bound ovomucoid. Finally, tetramethylbenzidine (TMB) is added to each well as enzyme substrate and, after color development, the enzymatic reaction is stopped with sulfuric acid. The yellow product formed is measured at 450 nm, and it is proportional to the amount of ovomucoid present in the sample.

CONTENTS AND COMPOSITION

A. Concentrated Washing Buffer. 60 mL. Phosphate buffered saline.
B. Concentrated Dilution Buffer. 2 x 120 mL. Tris buffer. Dyed red.
C. Conjugate. 15 mL. Peroxidase conjugated antibody directed against ovomucoid. Dyed red.
D. Substrate. 15 mL. 3,3',5,5'-tetramethylbenzidine (TMB).
E. Stop Solution. 15 mL. Sulfuric acid 0.5 mol/L.
M. Microplate. 12 strips of 8 wells each coated with anti-ovomucoid antibodies.
S1-S5. Ovomucoid Standards. 5 x 4.0 mL. Concentration: 0, 0.4, 1, 4 and 10 mg/L (ppm). Dyed red.

STORAGE AND STABILITY

Store at 2-8 ºC. Each component is stable until the expiry date marked in the label. Liquid components are stable once opened until the expiry date marked in the label if they are stored at the recommended temperature, well closed and care is taken to prevent contamination during their use.

Indications of deterioration:

− Reagents: presence of particulate material, turbidity.
− Microplate: rips on the plastic bag, macroscopic defects like scratches on the base of the well.

ADDITIONAL MATERIALS REQUIRED (NOT PROVIDED)

− Moist chamber.
− Multitip aspirator or automatic washing equipment for microplates.
− Microplate reader or photometer with microcuvette, with a ±10 nm filter.
− All reagents and materials required for the samples treatment are not provided.

REAGENT PREPARATION

Washing Buffer. Dilute Concentrated Washing Buffer (A) with distilled water in the proportion 1/10. Mix thoroughly. Stable 4 weeks at 2-8°C.

Dilution Buffer. Dilute Concentrated Dilution Buffer (B) with distilled water in the proportion 1/10. Mix thoroughly. Stable 1 week at 2-8°C.

All other reagents are provided ready to use.

Solutions A, B or E may precipitate upon the cold storage. Warm up to 37°C and mix to dissolve before using.

PRECAUTIONS

− The Stop Solution contains sulfuric acid. Do not allow the reagent to get into contact with the skin.
− Avoid contact of all biological materials with skin and mucous membranes.
− Do not pipette by mouth.
− Do not eat, drink, smoke, store or prepare foods, or apply cosmetics within the designated work area.
− TMB is toxic by inhalation, in contact with skin and if swallowed; observe care when handling the substrate.
− Do not use components past expiration date and do not intermix components from different serial lots.

SAMPLE TREATMENT

A homogeneous sample has to be obtained from a representative part of the food. For allergic persons the consumption of egg white represents a critical problem.

1. Sample (5 g) is ground and pulverised in a mortar, impact mill, etc. into a fine homogeneous compound (Note 1).
2. Dilute 1.0 g (or 1.0 mL, liquids) of the homogeneous compound with 20 mL (19 mL for liquids) of Dilution Buffer and incubate for 15 min in a water bath at 60°C. Shake the suspension every two minutes.
3. Centrifuge the suspension for 10 minutes at 2000 x g. Separate the supernatant from the precipitate completely. Filter if necessary.

PROCEDURE

Allow all the reagents and microwells warm up to room temperature. Duplicate determinations are recommended.

1. Open the Microplate package (M) and take out the required amount of wells (Note 2).
2. Pipette 100 µL of the standards (S1-S5) and treated samples into the wells of the plate.
3. Incubate (Note 3) for 20 minutes at room temperature (20-25°C).
4. Aspirate or discard the contents and wash the wells 3 times with 300 µL of Washing Buffer (Note 4).
5. Pipette 100 µL of Conjugate (D) to all wells.
6. Incubate for 20 minutes at room temperature (20-25°C).
7. Aspirate or discard the contents and wash the wells 3 times with 300 µL of Washing Buffer.
8. Pipette 100 µL of Substrate (E) into all wells.
9. Incubate for 20 minutes at room temperature (20-25°C).
10. Pipette 100 µL of Stop Solution (F) into all wells and let stand for 5 minutes at room temperature (Note 5).
11. Read the absorbance of the contents of each well at 450 nm (Note 6). The color is stable for at least 30 minutes.

CALCULATIONS

Plot the absorbance values (mean values of the duplicates) for each standard on the Y axis versus the ovomucoid concentrations on the X axis. The concentration of egg white proteins (ovomucoid) in samples is calculated by interpolating the absorbance in the calibration curve (recommended curves: 4-parameter, cubic spline, one site-hyperbola).

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 every new test.

<table>
<thead>
<tr>
<th>Ovomucoid mg/L (ppm)</th>
<th>% binding</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>74</td>
</tr>
<tr>
<td>1</td>
<td>34</td>
</tr>
<tr>
<td>0.4</td>
<td>17</td>
</tr>
<tr>
<td>0</td>
<td>3</td>
</tr>
</tbody>
</table>
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 treatment procedure is already considered.

**METROLOGICAL CHARACTERISTICS**

- Detection limit: 0.05 ppm.
- Quantification limit: 0.4 ppm. Due to the variety of sample matrices and their influence on the blank, it is recommended that results lower than the quantification limit be treated as negative.
- Linearity: The serial dilution of spiked samples (pasta, biscuit, cookies, sausage and chocolate) resulted in a dilution linearity of 93-112%.
- Linearity limit: For values higher than 10 ppm dilute the treated sample 1/10 with Dilution Buffer and repeat measurement. This additional dilution has to be considered when calculating the sample concentration.
- Precision: Intra-assay (4-9%), inter-assay (3-7%), inter-lot (5-11%).
- Specificity: Cross-reaction reactivities are:
  - Ovalbumin    0.25
  - Ovomucoid    0.14
  - Conalbumin   2.6
  - Lysozyme     < 0.0003
  - Chicken meat < 0.001

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, Macadamia nut, Rice, Pork, Soy, Walnut, Peach, Sunflower seeds/Pistachio, Sugar, Fish gelatin, Isinglass.

- Recovery: Mean recovery was determined by spiking samples with different amounts of ovomucoid:
  - Pasta    91%
  - Biscuit  83%
  - Cookies  85%
  - Sausage  98%
  - Dark chocolate 82%

**NOTES**

1. Due to high risk of cross-contamination all used instruments like applicator, mortar, glass vials etc. have to be cleaned thoroughly before and after each sample.
2. Store the unused wells into the plastic bag and reseal it, keeping the desiccant inside.
3. It is recommended to perform all incubations in a moist chamber in order to protect the microplate from evaporation and from light.
4. Manual rinsing or rinsing with automatic plate wash equipment can be performed. Washing Buffer should be completely removed from the wells. Care should be taken not to scratch the inner microwell surface along the procedure.
5. Stop Solution stops the enzyme reaction and must be pipetted into the wells at approximately the same rate as Substrate in step 8.
6. Some microplate readers allow bichromatic readings. In this case, use a secondary wavelength in the range 600-700 nm.

**BIBLIOGRAPHY**