Egg White in Food ELISA Kit

Catalog number: NR-R10191 (96 wells)

The kit is designed to detect the level of Egg White in food.

FOR **RESEARCH USE ONLY**. NOT FOR DIAGNOSTIC OR THERAPEUTIC PURPOSES

Important notes

Before using this product, please read this manual carefully; after reading the subsequent contents of this manual, please note the following specially:

- The operation should be carried out in strict accordance with the provided instructions.
- Store the unused strips in a sealed foil bag at 2-8°C.
- Always avoid foaming when mixing or reconstituting protein solutions.
- Pipette reagents and samples into the center of each well, avoid bubbles.
- The samples should be transferred into the assay wells within 15 minutes of dilution.
- We recommended that all standard, testing samples are tested in duplicate.
- **Using serial diluted sample is recommended for first test to get the best dilution factor.**
- If the blue color develops too light after 15 minutes incubation with the substrates, it may be appropriate to extend the incubation time (Do not over-develop).
- Avoid cross-contamination by changing tips, using separate reservoirs for each reagent.
- Avoid using the suction head without extensive wash.
- Do not mix the reagents from different batches.
- Stop Solution should be added in the same order of the Substrate Solution.
- TMB substrate is light-sensitive. Avoid prolonged exposure to the light.

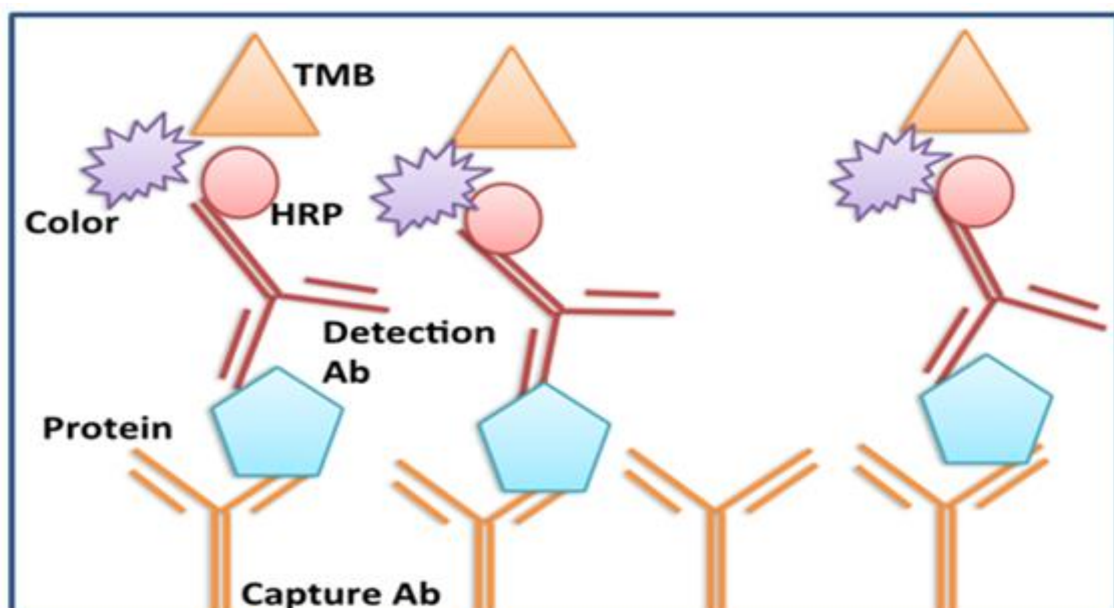
Intended use

The kit is used to quantify the Egg White in food. This assay has high sensitivity and excellent specificity for detection of Egg White. No significant cross-reactivity or interference between Egg White and analogues was observed.

Standard range	0–10 ppm
Sensitivity	0.05 ppm
Assay time	3.5 hrs
Validity	Six months
Store at	2-8 °C

Assay principle

This Egg White enzyme linked immunosorbent assay applies a technique called a quantitative sandwich immunoassay. The microtiter plate provided in this kit has been pre-coated with an antibody specific for Egg White (ovomucoid). Standards or samples are then added to the microtiter plate wells and Egg White if present, will bind to the antibody pre-coated wells. In order to quantitatively determine the amount of Egg White present in the sample, a standardized preparation of horseradish peroxidase (HRP)-conjugated antibody, specific for Egg White is added to each well to "sandwich" the Egg White immobilized on the plate. The microtiter plate undergoes incubation, and then the wells are thoroughly washed to remove all unbound components. Next, substrate solution is added to each well. The enzyme (HRP) and substrate are allowed to react over a short incubation period. Only those wells that contain Egg White and enzyme-conjugated antibody will exhibit a change in colour. The enzyme-substrate reaction is terminated by addition of a sulphuric acid solution and the colour change is measured spectrophotometrically at a wavelength of 450 nm.



Materials supplied

1	Microelisa Stripplate pre-coated with Egg White Ab	96 well
2	Standards*	5 vials
3	20 X Wash Solution	25 ml
4	Extraction/Diluent buffer	15 mlx 2
5	HRP-Conjugate Reagent	12 ml
6	TMB developing regent	12 ml
7	Stop Solution	12 ml
8	Closure plate membrane	2
9	Package insert	1

***Note: the concentrations of standards are as follow: 0; 0.4; 1; 4; 10 ppm**

Materials required but not supplied

- 37°C incubator.
- Standard plate reader capable of measuring absorbance at 450 nm.
- Adjustable pipettes and disposable pipette tips.
- Distilled water.
- Multi-channel pipettes, manifold dispenser or automated microplate washer.
- Absorbent paper.
- Materials used for sample preparation.

Sample collection and storage

- Pulverize a minimum of 5 g sample finely in a mortar, impact mill.
- 1 g of the homogenized mixture is suspended in 20 ml of pre-diluted extraction buffer. Incubated the suspension for 15 min in a preheated water bath at 60°C. To ensure good homogeneity, the samples should be shaken every two minutes.
- 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.
- If the results of a sample are out of the measuring range, further dilution with the pre-diluted extraction/Diluent buffer. The additional dilution has to be considered when calculating the concentration.

  Note:
- Novateinbio is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient amount of samples in advance.
- **Please predict the concentration before assaying. If values for these are not within the rang e of the standard curve, users must determine the optimal sample dilutions for their particular experiments.**
- If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.

- Owing to the possibility of mismatching between antigen from other resource and antibody used in our kits (e.g., antibody targets conformational epitope rather than linear epitope), some native or recombinant proteins from other manufacturers may not be recognized by our products.
- Influenced by the factors including cell viability, cell number and also sampling time, sample from cell culture supernatant may not be detected by the kit.
- Can't detect the samples containing NaN3, since NaN3 inhibits HRP (horseradish peroxidase) activity.
- Fresh samples without long time storage is recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.

Reagent Preparation

- Bring all kit components and samples to room temperature (18-25 °C) before use.
- Extraction/Diluent buffer (10x)– Dilute 15 ml of concentrated Extraction/Diluent buffer with 135 ml of deionized or distilled water to prepare 150 ml of working solution (1 ×).
- Wash Solution-Dilute 25ml of Wash Solution concentrate (20 ×) with 475ml of deionized or distilled water to prepare 500 ml of Wash Solution (1 ×).

Assay procedures

1. Prepare all the Standards before starting assay procedure (Please read Reagents Preparation). It is recommended that all Standards and Samples should be added in duplicate to the Microtiter Plate.
2. Assign standard wells, sample wells on the assay plate/strip. Add 100 µl of samples or standards to corresponding wells. Add 100 µl of diluent buffer to each well, mix well. Cover and incubate the plate for 1 hour at room temperature.
3. Wash the Microtiter Plate using one of the specified methods indicated below:
   Manual Washing: Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Fill in each well completely with diluted wash solution, and then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure five times for a total of FIVE washes. After washing, invert plate, and blot dry by hitting the plate onto absorbent paper or paper towels until no moisture appears.
   Note: Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame. Complete removal of liquid at each step is essential to good performance.
   Automated Washing: Wash plate FIVE times with diluted wash solution (350-400 µl /well /wash) using an auto washer. After washing, dry the plate as above. It is recommended that the washer be set for a soaking time of 10 seconds and shaking time of 5 seconds between each wash.
4. Add 100 µl of Conjugate to each well. Mix well. Mixing well in this step is important. Cover and incubate the plate for 30 minutes at room temperature.
5. Wash the plate as described above.
6. Add 100 µl of TMB developing reagent to each well. Cover and incubate 10 -20 minutes at room temperature. (Protect from light).
7. Add 100 µl Stop Solution to each well. Mix well.
8. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader immediately.

Result calculation

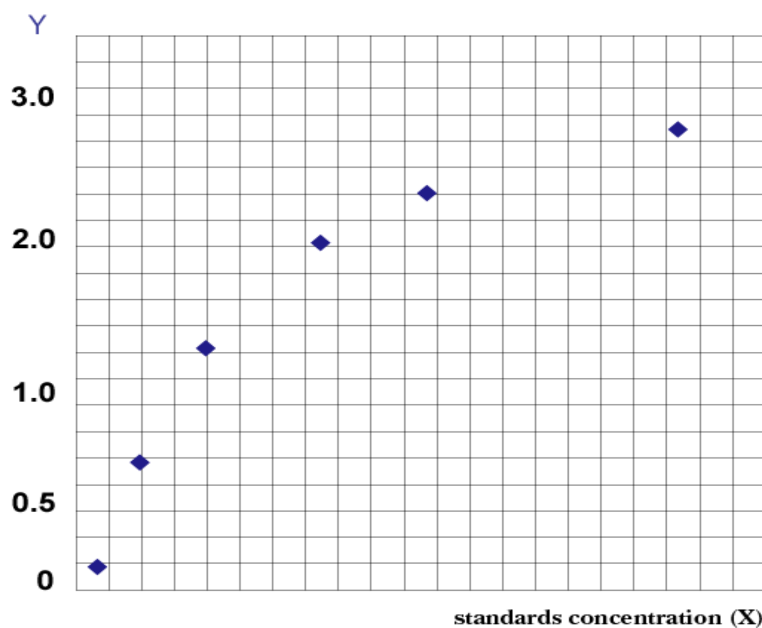
- This standard curve is used to determine the amount of an unknown sample. Construct a standard curve by plotting the average O.D. (450 nm) for each standard on the vertical (Y) axis

against the concentration on the horizontal (X) axis, and draw a best fit curve through the points on the graph.

- First, calculate the mean O.D. value for each standard and sample. All O.D. values, are subtracted by the mean value of the blank control before result interpretation. Construct the standard curve using graph paper or statistical software.
- To determine the amount in each sample, first locate the O.D. value on the Y-axis and extend a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the corresponding concentration.
- Any variation in operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in result. Each user should obtain their own standard curve.

Typical data

This standard curve is for demonstration purpose only. A standard curve must be run with each assay.



Background

It has been recognized that many proteins of egg white are 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.

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