INSTRUCTION FOR USE

SENSI*Spec* ELISA Egg White 96/48 Tests

(Cat. nr. HU0030007 / HU0030031)

Enzyme Immunoassay for the Quantitative Determination of Egg White Proteins in Food

Sensitivity (Egg White)	0.05 ppm
Recovery	82 - 98%
Incubation Time	60 min

1. 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 per-sons 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. 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.

The **Gold Standard Diagnostics 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.

2. PRINCIPLE OF THE TEST

The **Gold Standard Diagnostics 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 colour development is inhibited by the addition of a stop solution, and the colour turns yellow. The yellow colour is measured photometrically at 450 nm. The concentration of ovomucoid and with this the concentration of egg white is directly proportional to the colour intensity of the test sample.

3. PRECAUTIONS

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 in¬duce foaming.
3. Once the assay has been started, all subse¬quent steps should be completed without interruption and within the recommended time limits.
4. Replace caps in all the reagents immedi¬ately 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 labora¬tory equipment used during the pro¬cedure (micro¬pipets, ELISA reader etc.).

4. HEALTH AND SAFETY INSTRUCTIONS

1) Do not smoke or eat or drink or pipet 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).

5. REAGENTS

The kit contains reagents for 96/48 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 12/6 strips with 8 break¬able wells each, coated with anti-ovomu-coid anti-bodies.
2. Egg white protein standards (0; 0.4; 1; 4; 10 ppm of egg white protein): 5 vials with 2.0 mL each, dyed red, ready-to-use
3. Conjugate (anti-ovomucoid-peroxidase): 15/7.5 mL, dyed red, ready-to-use.
4. Substrate Solution (TMB): 15 mL, ready-to-use.
5. Stop Solution (0.5 M H2SO4): 15 mL, ready-to-use.
6. Extraction and sample dilution buffer (Tris): 2/1 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.

6. ADDITIONAL INSTRUMENTATION AND REAGENTS (NOT PROVIDED)

6.1. Instrumentation

- 100 - 1000 µL micropipets
- Volumetric flask
- Analytical balance
- Mortar, mixer
- Water bath
- Centrifuge
- ELISA reader (450 nm)

6.2. Reagents

- Double distilled water

7. SAMPLE PREPARATION

Due to high risk of cross-contamination all applied in-struments 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.

The following sample preparation should be applied for solid of samples:

1) 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 preheated 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.

The following sample preparation should be applied for liquid samples:

- 1 mL of liquid sample is diluted in 19 mL of **pre-diluted** extraction and sample dilution buffer.
- Afterwards the suspension is incubated for 15 min in a preheated water bath at 60°C.
- To ensure good homogeneity, the samples should be shaken every two minutes.
- The process is continued at point 3 of solid sample extraction process.

Attention:

- *Do not shake the final extract to prevent from resuspension.*
- *If after centrifugation a third layer at the top ap-pears due to a high fatty matrix, only the middle aqueous phase should be applied to the wells.*

8. 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 pipetted 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 re-commended.

The procedure is according to the following scheme:

1) Prepare samples as described above.
2) Pipet 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: Dis¬card the contents of the wells (dump or aspi¬rate). Pipet 300 µL of diluted washing solu¬tion 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. Insuf¬fi¬cient

washing will result in poor precision and falsely ele¬vated absorbencies.

5) Pipet 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) Pipet 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 colour will turn yellow upon addition.
11) After thorough mixing, measure absorbance at 450 nm (reference wavelength 620 nm), using an ELISA reader. The colour is stable for 30 minutes.

9. CALCULATION OF 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 ref¬erence stan¬dard against its concentration in ppm on semi-log graph paper with the op¬tical density on the ver¬tical (y) axis and the concentration on the hori¬zon¬tal (x) axis. Alternatively the evaluation can be carr¬ied out by software. In this case the 4-para¬me¬ter method should be preferred.

3. Using the mean optical density value for each sample, determine the corresponding concentra¬tion of egg white protein in ppm from the standard curve. De¬pending on experi¬ence and/or the avail¬ability of computer ca¬pability, other methods of data re¬duc¬tion may be employed.

The determined amount of egg white protein can be used to calculate the amount of the corresponding whole egg powder (NIST 8445) by multiplying with factor 4.35.

10. TYPICAL STANDARD VALUES

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

11. PERFORMANCE

11.1. Sensitivity

The limit of detection (LOD) of the **SENSI*Spec* ELISA Egg White test** is 0.05 ppm.
The limit of quantification (LOQ) of the **SENSI*Spec* ELISA Egg White test** is 0.4 ppm.

As matrices can have variable influence on the LOD in specific cases, and the range of matrices that was tested is of course limited, the end user if needed may evaluate its own LOD values depending on the matrices to be analyzed.

Alternatively, any results below LOQ should be just reported quantitatively as "< LOQ".

11.2. Cross-reactivity

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

Adzuki bean	Dill	Peach
Almond	Duck, raw	Peanut
Apricot	Ewe's milk	Pecan
Barley	Fennel	Pepper, black
Bean, white	Fenugreek	Pine seed
Beef, cooked	Fish gelatin	Pistachio
Beef, raw	Flaxseed	Plum
Bovine gelatin	Garden cress	Poppy
Brazil nut	Garlic, fresh	Pork
Buckwheat	Garlic, granulated	Potato
Cabbage, white	Ginge, ground	Prawn cooked
Caraway seeds	Ginger, fresh	Prawn, raw
Cardamom	Gliadin	Pumpkin seed
Carob gum	Goat's milk	Radish
Carrot	Guar gum	Rapeseed
Cashew	Gum arabic	Rice, brown
Cayenne	Hazelnut	Rice, white
Celery	Horseradish	Rye
Cherry	Kidney bean	Saccharose
Chestnut	Kiwi	Sesame
Chia seeds	Lamb	Shrimps
Chicken, cooked	Leek	Soy flour
Chickpea	Lentil	Soy lecithin
Chili	Lupin	Split pea
Cinamon	Macadamia	Sunflower seed
Clove	Mustard, yellow	Thyme
Cocoa	Nutmeg	Tofu
Coconut	Oats	Tomato
Cod	Octopus	Turkey,raw
Corn	Onion	Turmeric
Cow's milk	Paprika	Walnut
Cumin	Pea	Wheat

For the following commodities of the table above the results were between 0.5*LOQ and LOQ of the kit. So it cannot be completely excluded that these matrices may provide values above the LOQ in specific cases:

Chicken, cooked	Duck, raw	Thyme
Turkey, raw		

The following cross-reactions were determined:

Ovalbumin	0. 25%
Ovomucoid	614%
Conalbumin	2.6%
Lysozyme	<0.0003%
Chicken, raw	0.00006%

11.3. Precision

Intra-assay Precision	4 - 9%
Inter-assay Precision	3 – 7%

11.4. Linearity

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

11.5. 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%

Note: *The industrial process of food transformation has an impact on proteins which might affect testing performance. For this reason, recovery/cross reactivity may be altered for heat treated samples.*

12. LIABILITY

Gold Standard Diagnostics Budapest shall not be liable for any damages to the customer caused by the improper use of the kit and for any action undertaken as a consequence of the results.

Gold Standard Diagnostics Budapest shall not be liable for the unsafe use of the kit out of the current European safety regulations.