Chicken Egg White proteins ELISA Kit

Enzyme Immunoassay for the quantitative determination of Egg White proteins in food

Catalog number: ARG80796

TABLE OF CONTENTS

MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: No. 22, Ln. 227, Gongyuan Rd., Hsinchu City 300, Taiwan

Phone: +886 (3) 562 1738

Fax: +886 (3) 561 3008

Email: info@arigobio.com

INTRODUCTION

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 per-sons must strictly avoid the consumption of eggs or egg containing food. Non-declared addition of egg in food is hazardous for allergic people. Crosscontamination, most-ly in consequence of the production pro-cess is often noticed. The chocolate production pro-cess is a repre-sentative example. For the detection of egg white protein residues, sensitive detection systems are required.

PRINCIPLE OF THE ASSAY

This assay employs the sandwich quantitative enzyme immunoassay technique. An antibody specific for ovomucoid has to be bound onto a pre-coated microtiter plate. Egg White 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 color. The color development is inhibited by the addition of a stop solution, and the color turns yellow. The absorbance is proportional to the concentration of ovomucoid. A standard curve is constructed by plotting absorbance values against concentrations of standards, and concentrations of unknown samples are determined using this standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	12 strips x 8-well	4°C
HRP-antibody Conjugate	15 ml (ready to use)	4°C
Standards (0, 0.4, 1, 4, 10 ppm)	5 X 1 ml (ready to use)	4°C
10x Extraction Buffer	2 X 120 ml	4°C
10x Wash Buffer	60 ml	4°C
TMB substrate	15 ml	4°C (Protect from light)
STOP solution	15 ml	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Briefly spin down the HRP-Antibody conjugate before use.
- If crystals are observed in the 10X Wash buffer and Extraction Buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- Samples contain azide cannot be assayed.

SAMPLE COLLECTION & STORAGE INFORMATION

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. Tropomyosin could adhere to different surfaces. 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 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 and sample dilution buffer. Afterwards the suspension is incubated for 15 min in a preheated water bath at 40°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 40°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.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 10X wash buffer into distilled water to yield 1X wash buffer.
- 1X Extraction Buffer: Dilute 10X Extraction buffer into distilled water to yield 1X Extraction buffer.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 100 µl of standards and samples in duplicate into wells.
3. Incubate for 20 minutes at RT.
4. Aspirate each well and wash, repeating the process 2 times for a total 3 washes. Wash by filling each well with 1X wash buffer (350 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
5. Add 100 µl of HRP-Antibody Conjugate into each well. Incubate for 20 minutes at RT.
6. Aspirate and wash well as step 4.
7. Add 100 µl of TMB mixture to each well. Incubate for 20 minutes at room temperature in dark.
8. Add 100 µl of Stop Solution to each well.
9. Read the OD with a microplate reader at 450 nm immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

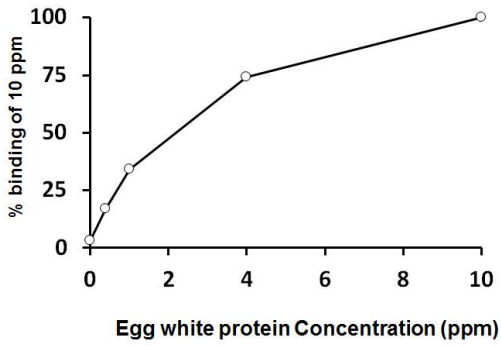
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The limit of detection (LOD) of the Egg White test is 0.05 ppm.

The limit of quantification (LOQ) of the 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.

Specificity

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

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

Reagent	Cross-reactivity (%)
Ovalbumin	0.25
Ovomucoid	614
Conalbumin	2.6
Lysozyme	< 0.0003
Chicken meat	< 0.001

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 4-9% and inter-assay precision was 3-7%.

Recovery

Mean recovery was determined by spiking samples with different amounts of Egg White:

Pasta	91%
Bicuit	83%
Cookies	85%
Sausage	98%
Dark chocolate	82%