Chicken Lysozyme ELISA Kit

Enzyme Immunoassay for the quantitative determination of Chicken Lysozyme in food

Catalog number: ARG80805

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, ovomucoid and livetin, lysozyme represents an important allergen. Primarily lysozyme is used as a preservative in wine and cheese industry. For allergic persons the consumption of lysozyme 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, lysozyme allergic per-sons must strictly avoid the consumption of lysozyme containing food. Non-declared addition of lysozyme in food is hazardous for allergic people. Cross-contamination, most-ly in consequence of the production pro-cess, is also problematic. Since July 1, 2012 the European Union requests allergen labeling for wine if milk or egg proteins are used during the production and are still present at a detection level of 0.25 mg/L or greater. Thus for the detection of lysozyme residues, sensitive assay systems are required.

The Lysozyme ELISA kit represents a highly sensitive detection system for lysozyme and is particularly capable of the quantification of lysozyme residues in wine and cheese.

PRINCIPLE OF THE ASSAY

This assay employs the sandwich quantitative enzyme immunoassay technique. An antibody directed against Lysozyme is bound on the surface of a microtiter plate. Lysozyme 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 Lysozyme proteins 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 yellow color is measured at 450 nm. The concentration of Lysozyme is directly proportional to the color intensity of the test sample.

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 0-4 (0, 25, 50, 100, 250 ppb)	5 X 2 ml (ready to use)	4°C
10x Extraction and sample dilution buffer	2 X 120 ml	4°C
10x Wash Buffer	60 ml	4°C
TMB substrate	15 ml (ready to use)	4°C (Protect from light)
STOP solution	15 ml (ready to use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- NaCl (if applicable)
- 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.
- If crystals are observed in the 10X Wash buffer, Extraction Buffer and Sample diluent buffer, warm to 37°C until the crystals are completely dissolved.
- Prior to beginning the assay procedure, bring all reagents to room temperature (20-25°C).
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Once the assay has been started, all subsequent steps should be completed without interruption and within the recommended time limits.
- Replace caps in all the reagents immediately after use. Do not interchange vial stoppers.
- 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.

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. Hazelnut proteins adhere very strongly to different surfaces. In certain cases they can resist a common dishwasher cleaning. To identify possible cross-contamination caused by previous extractions it is strongly recommended to note the sequence of the extractions.

A. The following sample preparation should be applied for wine samples:

1. 1 mL of the wine sample is diluted in 19 mL of 1X Extraction and sample dilution buffer.
2. 100 µL of the wine solution are applied per well. If the results of samples are out of the measuring range, further dilute the samples with the 1X Extraction and sample dilution buffer and re-assay. The additional dilution has to be considered when calculating the concentration. (The standards were designed for wine samples, so if there was no additional dilution, the concentration of Lysozyme in wine could be calculated from the OD of standard curve directly).

B. The following sample preparation should be applied for cheese and all kinds 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 10 mL of NaCl

contained 1X Extraction and sample dilution buffer (per 100 mL 1X Extraction and sample dilution buffer add 10g NaCl). 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 2000xg. If it is not possible to separate the supernatant from the precipitate completely, the suspension should be filtrated if necessary.
4. Dilute 100 µL of supernatant in 900 µL 1X Extraction and sample dilution buffer (without NaCl).
5. 100 µL of step.4 solution are applied per well. In case of using this extraction process for cheese or other samples than wine, the determined concentration has to be multiplied by 5 in order to get the sample concentration.
6. If the results of samples are out of the measuring range, further dilute the samples with the 1X Extraction and sample dilution buffer and re-assay. The additional dilution has to be considered when calculating the concentration.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 10X wash buffer into distilled water to yield 1X wash buffer.
- 1X Extraction and Sample diluent buffer: Dilute 10X Extraction and Sample diluent buffer into distilled water to yield 1X Extraction and Sample diluent 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 prepared 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 (300 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. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbencies.
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. Cover the plate and 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 (optional: read at 620 nm as reference wavelength). The color is stable for 30 minutes, it is recommended read the OD within 30 min.

CALCULATION OF RESULTS

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

2. Using semi-log or 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.

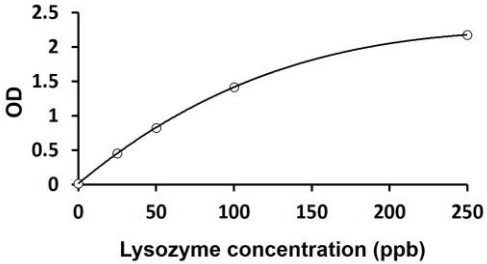
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results. The diluted samples must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

5. The ready-to-use standards are prepared for a direct determination of the concentrations of lysozyme in wine samples. The dilution of wine samples in the extraction process as described in the above stated sample preparation procedure for wine samples is already considered. In case of using the extraction process for cheese or other samples than wine, the determined concentration has to be multiplied by 5 in order to get the sample concentration.

6. If the calculated concentration is higher the highest standards, further dilute the samples with the 1X Extraction and sample dilution buffer and re-assay. The additional dilution has to be considered when calculating the concentration. .

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 Lysozyme test is 2 ppb.

The limit of quantification (LOQ) of the Lysozyme test is 25 ppb.

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

Specificity

The following cross reactions were determined:

Reagent	Cross-reactivity (%)
Ovalbumin	< 0.0001
Ovomucoid	< 0.0001
Conalbumin	< 0.0001
Egg White Protein (total)	2.2
Non Fat Dry Milk	0
Fish	0
Bovine Gelatin	0

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 2-6% and the CV value of inter-assay precision was 2-3%

Recovery

Wine	99%
Cheese	85%

Linearity

The serial dilution of spiked samples (white wine, red wine & cheese) resulted in a dilution linearity of

87%- 98%