Derivatized histamine ELISA Kit

Enzyme Immunoassay for the quantification of Histamine in food

Catalog number: ARG80803

For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Fish meal that has been produced from materials which has been allowed to degrade prior to being processed can contain high levels of histamine and can be toxic. Elevated histamine levels (1,000 ppm) can cause gizzard erosion and black vomit in poultry. Histamine testing in fresh fish is a possible control strategy that can be used by seafood processors in their HACCP program to address the hazard of scombrotoxin formation. Histamine is a product of decomposition of histidine caused by the growth of certain bacteria in seafood. The amount of the amine that forms is a function of bacterial species, the temperature and time of exposure, and may exceed 1,000 ppm (mg/kg). Fish containing high levels of histamine has been associated with many examples of poisoning commonly referred to as "scombroid poisoning," a major health problem for consumers. Scombrotoxic fish usually contains levels of histamine in excess of 200 ppm but such fish may be randomly dispersed within a lot. For large fish, histamine is found at variable levels even within individual fish. Quality control measures designed to minimize the occurrence of scombrotoxic fish require the determination of histamine levels in the range of approximately 10 to 200 ppm. Good quality fish contain less than 10 ppm histamine, a level of 30 ppm indicates significant deterioration, and 50 ppm is considered to be evidence of definite decomposition. The defect action level (DAL), the level at which regulatory actions are taken for histamine is 50 ppm.

PRINCIPLE OF THE ASSAY

This assay employs the competitive enzyme immunoassay technique. Histamine is bound to the solid phase of the microtiter plate. Acylated

histamine and solid phase bound histamine compete for a fixed number of antiserum binding sites. When the system is in equilibrium, free antigen and free antigen-antiserum complexes are removed by washing. The antibody bound to the solid phase histamine is detected by anti-goat/peroxidase. The substrate TMB/peroxidase reaction is monitored at 450 nm. The amount of antibody bound to the solid phase histamine is inversely proportional to the histamine concentration of the sample.

MATERIALS PROVIDED & STORAGE INFORMATION

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

Component	**Quantity**	**Storage information**
Reaction Plate	96 wells	4°C, ready for use
Histamine coated microtiter strips	12 x 8 wells	4°C, ready for use
Standard A-F	6 x 4 ml	4°C, ready for use
Control 1	4 ml	4°C, ready for use
Control 2	4 ml	4°C, ready for use
Acylation Buffer	2 x 12 ml	4°C, ready for use
Acylation Reagent	2 x 1.5 ml	4°C, ready for use
Enzyme-conjugated Antibody	12 ml	4°C, ready for use
Histamine antiserum	6 ml	4°C, ready for use
50X Wash buffer	20 ml	4°C
TMB substrate	12 ml	4°C (Protect from light)
STOP solution	12 ml	4°C, ready for use

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 50X Wash 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.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Fish meal–Suspend 1 g of fish meal in 200 ml of distilled water and stir for 15 minutes. Pipette 1 ml of the suspension into an Eppendorf-tube or similar centrifugation device and centrifuge for 5 minutes at maximum

speed. Take 20 µl of the supernatant and dilute it with 20 ml of distilled water (for this dilution step, do not use any glass ware!). Use 100 µl for the acylation!

Fresh fish, sausage (processed, smoked or fermented meats), cheese - Homogenize 10 g of fresh fish (sausage, cheese) in 90 ml of distilled water for 1–2 minutes by use of a house-hold food mincer. Pipette 1 ml of the suspension into an Eppendorf-tube or similar centrifugation device and centrifuge for 5 minutes at maximum speed. Remove lipid layer by suction! Take 20 µl of the supernatant and dilute it with 10 ml of distilled water (for this dilution step, do not use any glass ware!). Use 100 µl for the acylation!

Milk- Pipette 10 µl of milk into a centrifugation tube. Add 50 µl of precipitator. Vortex mix, incubate for 5 minutes and add 2 ml of 0.1 N hydrochloric acid (HCl). Centrifuge for 5 minutes at 3,000 x g and remove the lipid layer by suction. Use 100 µl for the acylation!

Wine, champagne-Dilute 20 µl with 10 ml of distilled water (for this dilution step, do not use any glass ware!). Use 100 µl for acylation!

Sample	Fish meal	Fresh fish, sausage, cheese	milk	wine, champagne
Dilution Factor	200000	5000	200	500

Cautions:

- Avoid excess of acid: excess of acid might excessed the buffer capacity. A pH >7 during the extraction is mandatory.
- Prevent catecholamine degradation by adding preservatives to the sample.
- Avoid chaotropic chemicals like perchloric acid. The high salt might reduce the recovery of Dopamine.

- Tissue sample can be homogenized in 0.01 N HCl in the presence of EDTA and sodium metabisulfite.
- Avoid samples contain substance with cis-diol structure.
- The used sample volume determines the sensitivity of this test.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 50X Wash buffer into distilled water to yield 1X Wash buffer.

ASSAY PROCEDURE

- **Acylation**

1. Pipette 100 µL of standards, controls and extracts into the respective wells of the Reaction Plate.
2. Add 25 µL of Acylation Reagent to all wells.
3. Pipette 200 µL of Acylation Buffer into all wells.
4. Incubate 15 minutes at RT (20-25°C) on a shaker (approx. 600 rpm)
   Alternative protocol without shaker: shake the plate shortly by hand and incubate for 15 min at RT.

- **Histamine ELISA**

1. Pipette 25 µL of the acylated standards, controls and samples into the wells of the Histamine Microtiter Strips.
2. Pipette 100 µL of the Histamine Antiserum into all wells.
3. Incubate 30 min at RT (20-25°C) on a shaker (approx. 600 rpm).
   Alternatively without shaker: shake the Histamine Microtiter Strips shortly by hand and incubate for 40 min at RT (20-25°C).

4. Aspirate each well and wash, repeating the process 3 times for a total 4 washes. Wash by filling each well with 1× 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. Pipette 100 μL of the Enzyme Conjugate into all wells.
6. Incubate for 10 min at RT (20-25°C) on a shaker (approx. 600 rpm). Alternatively without shaker: incubate for 20 min at RT (20-25°C).
7. Wash as according to step 4.
8. Add 100 μl of TMB Reagent to each well. Incubate for 15 minutes at room temperature in dark.
9. Add 100 μl of Stop Solution to each well.
10. 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.

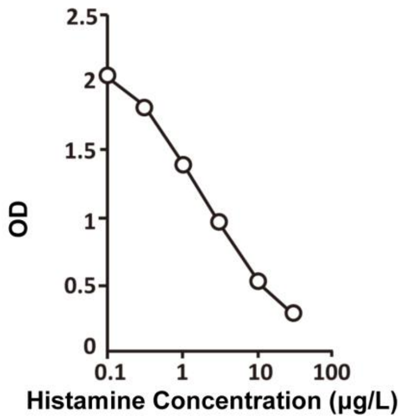
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

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 minimum detectable dose (MDD) of Histamine was 0.15 µg/L

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 9.15% and inter-assay precision was 6.8%.

Recovery

Milk: 95-146%

Wine: 87-107%

Fish: 95-113%

Cross Reactivity	Substance	Cross Reactivity (%)
		Histamine
	Histamine	100
	3-Methyl-Histamine	0.01
	Tyramine	< 0.001
	L-Phenylalanine	< 0.001
	L-Histidine	< 0.001
	L-Tyrosine	< 0.001
	Tryptamine	< 0.001
	5-Hydroxy-Indole-Acetic Acid	< 0.001
	Serotonin	< 0.001