Histamine ELISA Kit

Catalog Number KA1420

96 assays

Version: 46

Intended for research use only

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Introduction

Intended Use

Enzyme Immunoassay for the determination of Histamine in Fish Extract.

Background

Histamine, 4-(2-aminoethyl)imidazole (MW = 111), is a primary amine arising from the decarboxylation of the amino acid L-histidine. Therefore, histamine formed in foods is the result of the growth of bacteria that possess the enzyme histidine decarboxylase. Scombrotoxin poisonings have primarily been associated with the consumption of tuna, mahi mahi, and bluefish. The scombrotoxin formation that causes consumer illness is most closely linked to the development of histamine in these fish. However, a number of other species are also capable of developing elevated levels of histamine as a result of time/temperature abuse. Histamine is heat-stable and survives thermal processing. The quality of the commercial fishmeal and other related product is directly related to the histamine content of these products. The Histamine ELISA Kit is a simple screening system for histamine in fish.

Principle of the Assay

The enzyme immunoassay for histamine is based on the competition between the histamine to be assayed and the histamine-alkaline phosphatase conjugate, for binding to antibody directed against histamine, coated onto microtiter wells. The sample containing the histamine, and the histamine-alkaline phosphatase conjugate, when added to the microtiter wells, compete for binding to a limiting number of antibody sites. After incubation, each well is rinsed in order to remove non-bound components. The bound enzymatic activity is then measured by the addition of a chromogenic substrate. The intensity of the color developed is inversely proportional to the concentration of histamine in the sample. The concentration is estimated by comparison with standard histamine solution.

General Information

Materials Supplied

List of component

Component	Amount
96-well Microtiter plate (#S): Twelve strips of 8 detachable wells, coated with Anti-Histamine antibody.	96 (8x12) wells
Calibrator: 0, 2.5, 5.0 and 10.0 µg/mL of histamine.	0.9 mL x 4
Histamine-Alkaline Phosphatase conjugate (HTM-ALP) (#3)	10.5 mL
p-Nitrophenyl Phosphate (pNPP) substrate (#5). Ready to use.	10.5 mL
Wash Buffer (10x PBS-Tween) (#6). Dilute 10 fold with distilled or deionized water to 150 mL prior to use.	15 mL
Stop Solution (#7): 3 N NaOH.	5.5 mL

Storage Instruction

All reagents of the kit are stable, if stored at 2-8°C, until the expiration date stated on the kit.

Materials Required but Not Supplied

- ✓ Pipettors capable of delivering 50 µL and 100 µL.
- ✓ Microtiter plate reader (wavelength 405 nm).
- ✓ Plate washer or squeezable wash bottle.
- ✓ Timer.
- ✓ Absorbent paper towels.

Precautions for Use

Reagents are for in vitro research use only. Not for Diagnosis.

- ✓ Histamine adsorbs to glass; therefore employ only plastic pipettes and tubes.
- ✓ Do not mix reagents from different lots.
- ✓ If concentrations of histamine in the samples are high (100 µg/mL), dilute sample with 0.05 M Tris-HCl Buffer, pH 6.5 such that points fall in the middle range of the standard curve.
- ✓ Do not return unused reagents back into their original bottles.
- ✓ Samples tested should have a pH of 7.0 (± 1.0). Excessive alkaline or acidic conditions may affect the test results.
- ✓ The stop solution contains NaOH. Do not allow to contact skin or eyes. If exposed, flush with water.
- ✓ Dispose of all materials, containers and devices in the appropriate receptacle after use.

Assay Protocol

Sample Preparation

1. Weigh 2 g of representative sample into a 30 mL extraction tube that is filled with 20 mL of 0.05 M Tris-HCl buffer, pH 6.5. Close the screw cap tightly.
2. Shake the tube continuously for one minute and set aside at room temperature. Shake it again briefly three more times at 5 minutes interval. Allow the contents to settle for ~2 min.
3. If necessary, dilute the supernatant from the above extract with 0.05 M Tris-HCl buffer, pH 6.5 to 1 in 10, and 1 in 50 before testing.

Assay Procedure

Let the components of the kit equilibrate to room temperature before use.

1. Carefully add 50 µL of standard or sample to the bottom of each well. Slightly tap the side of the strip holder to evenly distribute the sample.
2. Avoid touching the well with pipette tip and add 100 µL of HTM-ALP conjugate (#3) to each well. Slightly tap the side of the strip holder to properly mix the sample and enzyme conjugate.
3. Incubate at room temperature for 40 minutes.
4. After incubation, dispose the solution in the wells by inverting and shaking. Wash microtiter wells 3 times with wash buffer to remove the non-bound conjugate. Washing may be done manually as follows: use squeeze bottle to fill wells gently with wash buffer, dumping the wells between each wash by inverting and shaking. After the third wash, tamp holder with washed strips onto a piece of absorbent paper.
5. Add 100 µL of pNPP substrate (#5) to each well and incubate at room temperature for 20 min. To avoid contamination, place the needed amount of substrate into a test tube and only dispense from the tube itself.
6. Add 50 µL of Stop Solution (#7) to each well and tap the strip holder for proper mixing.
7. Read absorbance at 405 nm using ELISA reader.

Simplified Assay Procedure

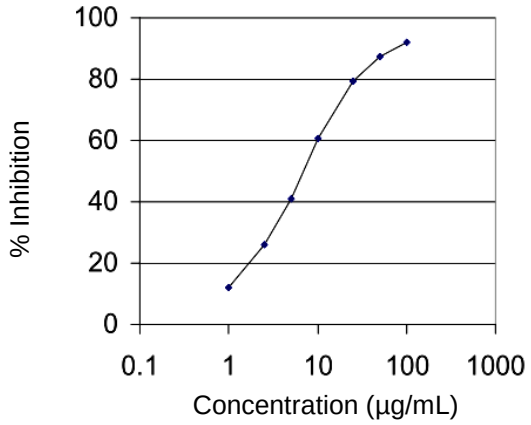
1. Add sample or standard (50 µL).
2. Add enzyme conjugate (100 µL). 40 min at RT.
3. Wash 3x.
4. Add pNPP (100 µL), wait for 20 min at RT.
5. Add stop solution (50 µL) and read at 405 nm.

Data Analysis

Calculation of Results

1. Calculation
   a. Average the absorbance (OD_S) for each standard concentration of histamine including 0 µg/mL (OD_0).
   b. % of Inhibition = 100 - (OD_S / OD_0) x 100
2. Plot values of % of Inhibition, step 1 (b), against their corresponding concentrations on Log_{10} paper.
3. Calculate histamine concentration in the sample by interpolation and multiply by the sample's dilution factor to obtain the actual quantity of histamine.

✓ Histamine Inhibition curve



Performance Characteristics

✓ Cross reactivity

By the assay, the following compounds tested at the stated levels are found to give results not greater than a level of 2.5 µg/mL of histamine.

Compound	Conc. (µg/mL)	% Inhibition
Histidine	1000	<5
Serotonin	1000	<5
Spermidine	500	<10
Putrescine	500	<10
Spermine	500	<10
Tyramine	500	<10
Cadaverine	500	<10

There is the possibility that other substances and/or factors not listed above may interfere with the test.

Resources

Plate Layout

12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								
	A	B	C	D	E	F	G	H