

Importance of Histamine Determination

Histamine is the agent that causes scombroid poisoning, a foodborne chemical intoxication. Scombrosia is among the top three seafood illness reported in the United States. Many instances of histamine poisoning, especially in recreational-caught fish, go unreported to health officials. Large amounts of histamine can be formed in these fish if they are poorly handled and not stored at proper temperature. The symptoms of histamine poisoning can include dizziness, headache, facial swelling and flushing, nausea, abdominal cramping, diarrhea, and difficulty in swallowing. Most victims recover within 24 hours especially with the aid of antihistamines. However, individuals sensitive to histamine can suffer for longer periods of time.

Fish contain relatively large amounts of the amino acid histidine in their muscle. After death, histamine forming bacteria that occurs naturally in fish (or added during handling) can transform the histidine to histamine by carboxylation. Therefore, histamine formed in foods is the result of the growth of bacteria that possess the enzyme histidine decarboxylase. Histamine is heat stable and survives thermal processing. The quality of fish, commercial fishmeal and other related products are directly related to the histamine content of these products.

In the USA, Histamine is regulated by the FDA in accordance to the implementation of the HACCP principles in the seafood industry. The guidelines for histamine in fish, and fishery products is 5 mg/100 g (50 ppm).

The ABRAXIS® Histamine ELISA Kit provides a system screening system for histamine in fish and fish products

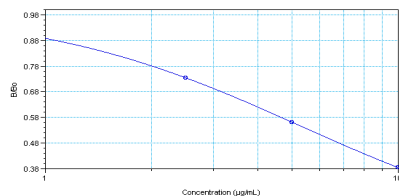
Performance Data

Test sensitivity: The detection limit for this assay is 0.8 ppm ($\mu\text{g/mL}$)

Test reproducibility: Coefficients of variation (CVs) for calibrators: <10%, for samples: <15%.

Selectivity: Other compounds tested at the stated levels were found to give results not greater than 1.0 $\mu\text{g/mL}$ of histamine.

Compound	Conc. ($\mu\text{g/mL}$)	Inhibition (%)
Histidine	1000	< 5
Serotonin	1000	<5
Spermidine	500	<10
Putrescine	500	<10
Spermine	500	<10
Tyramine	500	<10
Cadaverine	500	<10



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ABRAXIS® Histamine ELISA Microtiter Plate

Enzyme-Linked Immunosorbent Assay for the Determination of Histamine
in Fish Samples

Product No. 515168

1. General Description

The ABRAXIS® Histamine ELISA is an immunoassay for the quantitative and sensitive detection of Histamine in fish extracts. If necessary, positive samples can be confirmed by HPLC, or other conventional methods

2. Safety Instructions

The stop solution contains diluted sodium hydroxide. Avoid contact of the stopping solution with skin and mucous membranes. If these reagents come in contact with the skin, wash with water.

3. Storage and Stability

The ABRAXIS® Histamine ELISA should be stored in the refrigerator (2-8°C). Solutions should be allowed to reach room temperature (20-25°C) before use. Reagents may be used until the last day of the month as indicated by the expiration date on the box. Consult state, local and federal regulations for proper disposal of all reagents.

4. Test Principle

The test is a direct competitive ELISA that allows the detection of Histamine. It is based on the recognition of Histamine by specific antibodies. Histamine, when present in a sample and a Histamine-AP analogue compete for the binding sites of rabbit anti-Histamine antibodies that have been immobilized onto the microtiter wells. After a washing step and addition of the substrate solution, a color signal is generated. The intensity of the yellow color is inversely proportional to the concentration of the Histamine present in the sample. The color reaction is stopped after a specified time and the color is evaluated using an ELISA reader. The concentrations of the samples are determined by interpolation using the calibration curve constructed with each run.

5. Limitations of the ABRAXIS® Histamine ELISA, Possible Test Interference

Many organic and inorganic compounds commonly found in samples have been tested and found not to interfere with this test. However, due to the high variability of compounds that might be found in samples, test interferences caused by matrix effects cannot be completely excluded. Mistakes in handling the test also can cause errors. Possible sources for such errors can be:

Inadequate storage conditions of the test kit, wrong pipetting sequence or inaccurate volumes of the reagents, too long or too short incubation times during the immune and/or substrate reaction, extreme outside temperatures (lower than 10°C or higher than 35°C) during the test performance. The assay procedure should be performed away from direct sun light.

The ABRAXIS® Histamine ELISA kit provides screening results. As with any analytical technique (GC, HPLC, etc.) samples requiring some regulatory action should be confirmed by alternative methods

6. Working Instructions

A. Materials Provided

1. Microtiter plate coated with anti-Histamine antibody. Twelve strips of 8 detachable wells.
2. Calibrator 0 (Negative Control), containing 0 µg/mL Histamine, 1.2 mL.
3. Calibrators (3), containing Histamine with concentrations of: 2.5, 5.0, 10.0 µg/mL (ppm), 0.9 mL.
4. Histamine-Alkaline Phosphatase (AP) Enzyme Conjugate, 10.5 mL
5. Extract Solution/Sample Diluent (10X), 100 mL. Use to extract samples and to dilute samples with concentration above 100 µg/mL (ppm).
6. Wash Solution 10X Concentrate, 15 mL
7. Substrate/Color Solution (pNPP), 10.5 mL
8. Stop Solution (3 N NaOH), 5.5 mL

B. Additional Materials (not delivered with the test kit)

1. Micro-pipettes with disposable plastic tips (50-250 µL)
2. Multi-channel pipette (50-250 µL) or stepper pipette with plastic tips (50-250 µL)
3. Reagent reservoir for multichannel pipettes
4. Microtiter plate washer (optional)
5. Microtiter plate reader (wavelength 405 nm)
6. 50 mL plastic extract tubes with caps
7. Overhead tube rotator or equivalent

C. Preparation and Extraction of Fish Samples

1. Weigh a 2.0 gm of a representative sample into a 50 mL plastic extraction tube.
2. Add 20 mL of the 1X Extraction solution, close tightly.
3. Shake the tube continuously for one minute, set aside at room temperature for 5 minutes. Shake again for 20 minutes.
4. Allow contents to settle for 3-5 minutes.
5. Sample is ready to test in the ELISA. Without disturbing the tube, use the supernatant (top portion) for assay. If necessary, dilute the supernatant with 1X Sample Diluent solution to 1:10 and 1:50 before testing.

The Histamine concentration contained in the samples is determined by multiplying the concentration of the extract by the dilution factor used. Highly contaminated samples outside the range of the curve should be diluted further and re-analyzed.

D. Test Preparation

Micro-pipetting equipment and pipette tips for pipetting the calibrators and the samples are necessary. We recommend using a multi-channel pipette or a stepping pipette for adding the enzyme conjugate, substrate solution, and the stop solution in order to equalize the incubations periods of the calibrator solutions and the samples on the entire microtiter plate. Please only use the reagents and calibrators from one package lot in one test, as they have been adjusted in combination. Read and understand the instructions and precautions given in this insert before proceeding.

1. Adjust the microtiter plate and the reagents to room temperature before use.
2. Remove the number of microtiter plate strips required from the foil bag. The remaining strips are stored in the foil bag and zip-locked closed. Store the remaining kit in the refrigerator (2-8°C).
3. The calibrators, control, enzyme conjugate, substrate and stop solutions are ready to use and do not require any further dilutions.
4. The wash solution is a 10X concentrated solution and needs to be diluted with deionized water. In a 0.25 L container dilute the 10X solution 1:10 (i.e. 15 mL of the 10X wash solution plus 135 mL of deionized water). The diluted solution is used to wash the microtiter wells.

5. The Extract/Sample Diluent solution is a 10X concentrated solution and needs to be diluted with deionized water. In a 1 L container dilute the 10X solution 1:10 (i.e. 100 mL of the 10X wash solution plus 900 mL of deionized water). The diluted solution is used to extract samples and to dilute extracts if necessary.

E. Working Scheme

The microtiter plate consists of 12 strips of 8, which can be used individually. The calibrators must be run with each test. Never use the values of calibrators that have been determined in a test performed previously.

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

Calibrators : 0, 2.5, 5, 10 ppm

Samples: Sam1, Sam2, Sam3, etc.

F. Assay Procedure

1. **Add 50 µL of the calibrator solutions, negative control or samples** into the wells of the test strips according to the working scheme given. We recommend using duplicates or triplicates.
2. **Add 100 µL of the enzyme conjugate solution** to the individual wells successively using a multi-channel pipette or a stepping pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a rapid circular motion on the benchtop for about 30 seconds. Be careful not to spill contents. **Incubate the strips for forty (40) minutes at room temperature.**
3. After incubation, remove the covering and vigorously shake the contents of the wells into a sink. **Wash the strips three times using the 1X washing buffer solution.** Please use at least a volume of 250 µL of washing buffer for each well and each washing step. Remaining buffer in the wells should be removed by patting the plate dry on a stack of paper towels. Alternatively, a squeeze bottle may be used to fill the wells between each wash.
4. **Add 100 µL of substrate/color solution** to the wells using a multi-channel pipette or a stepping pipette. The strips are incubated for 20 minutes at room temperature. Protect the strips from sunlight.
5. **Add 50 µL of stop solution** to the wells in the same sequence as for the substrate/color solution using a multi-channel pipette or a stepping pipette.
6. Read the absorbance at 405 nm using a microplate ELISA photometer within 15 minutes after stopping the reaction.

G. Evaluation

The evaluation of the ELISA can be performed using commercial ELISA evaluation programs (4-parameters, Logit/Log or alternatively point to point). For a manual evaluation, calculate the mean absorbance value for each of the calibrators. Calculate the %B/B₀ for each calibrator by dividing the mean absorbance value for each calibrator by the Zero Calibrator (Calibrator 0) mean absorbance. Construct a calibrator curve by plotting the %B/B₀ for each calibrator on a vertical linear (y) axis versus the corresponding Histamine concentration on horizontal logarithmic (x) axis on graph paper. %B/B₀ for controls and samples will then yield levels in ppb of Histamine by interpolation using the calibrator curve. The concentrations of the samples are determined using the constructed calibration curve (do not use a previously stored curve). Samples showing a lower concentration than the lowest calibrator (2.5 ppm) of Histamine are considered to be negative. Samples showing a higher concentration than the highest calibrator (10.0 ppm) must be diluted to obtain more accurate results