hygiena™

AlerTox® ELISA Histamine

Competitive ELISA test for the determination of Histamine in fish and wine.

REF KIT3065

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Contents

1. Introduction

AlerTox® ELISA Histamine test kit provides reagents for the quantitative analysis of Histamine in food products by ELISA.

Please do not modify the protocol in respect of the timings, the pipetting volumes, the type of buffers, the pH value of the buffers and the temperature. Any modification of the protocol as described before, will cancel the validation of the test system.

A pH adjustment is generally not necessary. Do not shake the plate during incubation. Do not use kit components after the expiration date.

Histamine is a biogenic amine, which is formed by enzymatical decarboxylation from the amino acid histidine. It occurs in the mast cells and basophilic white cells bound to heparin. In the course of a type I allergy, endogenic histamine from the mast cells and basophilic leucocytes is released after antigen binding to membrane-associated IgE, and typical allergic reactions appear. But histamine can also enter the human body via ingested food and drink, and can thus cause pseudo allergic food intolerances. Food intolerances, which are caused by increased histamine concentrations, are clinically characterized by rash, diarrhea, vomiting, nausea, itching, headache and asthma. The extent of the reaction is dependent on the ingested amount of histamine. Toxic histamine concentrations may arise by inappropriate handling or a disrupted cold chain. They can cause the so-called scombroid reaction, which appears after bacterial degradation of protein-rich food, especially fish from the *Scombridae* family. The US Food and Drug Administration has established an action level of 50 ppm for histamine in fish. According to EU Regulation 2073/2005 and further amendments, the limit for histamine in fish or fish products is 100 ppm. This limit is raised to 200 ppm if the fish or fish products have undergone enzymatic maturation in brine.

Thus, a monitoring of fish and fish products with respect to the concentration of histamine is obligatory. The AlerTox® ELISA Histamine is a sensitive detection system and is capable of rapid quantification of histamine concentrations in fish and wine.

2. Limits

Kit	Limit of Detection	Limit of Quantification
AlerTox® ELISA Histamine	0.3-0.7 ppm	2 ppm

3. Quantification ranges

Kit	Range
AlerTox® ELISA Histamine	72.0 - 24.0 – 12.0 – 6.0 – 2.0 – 0 ppm

4. Recovery (Tested in typical matrices)

Codfish 102 %	Trout 96%
Plaice 95%	Tuna 99%
Salmon 84%	Wine 97%

5. Cross-reactivity

Protein	Cross-reactivity
Histamine	100%
N-Acetylhistamine	0.02%
1-Methylhistamine	5%

6. Shelf life

12 months from date of production.

7. Tested matrices

Wine (red, rosé, white) and fish (trout, salmon, tuna, cod, plaice).

8. Tested non-cross-reactive matrices

Serotonin (0%)	Salmon (0%)
Histidine (0%)	Tuna (0%)
Wine (red, rosé, white) (0%)	Cod (0%)
Trout (0%)	Plaice (0%)

9. Results are measured as

Results of AlerTox® ELISA Histamine are measured as ppm of histamine.

10. General precautions

- Read this manual carefully before starting the test.
- The test must be performed by specialized and trained staff.
- Handle the test kit in accordance with good laboratory practices (GLP).
- Do not interchange reagents between kits of different lot numbers.
- Do not use reagents beyond the expiration date of the kit.
- The alteration of a reagent can cause inaccurate results.
- Do not exchange the vial caps.
- Use sterile pipette tips.
- Do not use solutions if they become cloudy or precipitate. The only exception is Washing Buffer 10x which may precipitate and must be completely dissolved by warming up at 37°C (99 °F) for 15 minutes before use.
- Use only distilled water for the dilutions of concentrated buffers.
- Substrate solution is light sensitive. Avoid exposure to direct light.

- Do not allow wells to dry completely.
- Handle any solution with gloves.
- During the sample extraction, avoid cross-contamination.
- Devices such as a blender must be cleaned after each sample preparation.
- All reagents must be rebalanced at room temperature (15 - 25 ºC/59 - 77 ºF) before use.
- Substrate Solution contains TMB, which is highly toxic if inhaled, ingested, or comes in contact with the skin. Please refer to the SDS.
- If you get in contact with toxic or irritating substances, rinse the affected skin area with plenty of water. Please refer to the SDS.
- Stop Solution contains sulphuric acid, which is corrosive. Please refer to the SDS.
- Avoid incubating on cold work benches.

11. Test principle

AlerTox® ELISA Histamine is based on the principle of a quantitative competitive ELISA. A histamine conjugate is bound on the surface of a microtiter plate. Samples or standards containing derivatized histamine and an antibody directed against histamine are added into the wells of the microtiter plate. Immobilized and free histamine compete for the antibody binding sites. After 5 minutes incubation at room temperature, the wells are washed with diluted washing solution to remove unbound material. A peroxidase conjugate directed against the histamine antibody is added into the wells and after another 5 minutes incubation, the plate is washed again. Then a substrate solution is added and incubated for 5 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 photometrically at 450 nm. The concentration of histamine is indirectly proportional to the color intensity of the test sample.

12. Supplied materials

The kit contains sufficient reagents for 96 determinations (including standards).

Description	Quantity
Breakable strips coated with histamine conjugate (8 wells each)	12 strips
Standards (72.0 - 24.0 – 12.0 – 6.0 – 2.0 – 0 ppm)	6x 4 mL
Reaction Solution	1x 3 mL
Neutralizing Solution	1x 15 mL
Anti-Histamine Antibody; blue	1x 6 mL
Conjugate Solution, red	1x 15 mL
Substrate Solution (TMB)	1x 15 mL
Stop Solution, containing H_2SO_4	1x 15 mL
Sample Dilution Buffer	2x 60 mL
Washing Solution 10x, blue	2x 60 mL

13. Storage advice

- All kit components should be kept at 2 – 8 °C (36 - 46 °F) in the dark. DO NOT FREEZE.
- Return all reagents to 2 – 8 °C (36 - 46 °F) in the dark immediately after use.
- The diluted Washing Solution concentrate can be used for 4 weeks, when stored at 2 – 8 °C (36 - 46 °F).
- The Sample Extracts are stable for at least 24 hours at 2 – 8 °C (36 - 46 °F), or frozen for longer storage.

14. Material required but not provided

- Multi-channel pipette 50-200 µL
- Sterile pipette tips
- Pipettes 10-100 µL, 100-1000 µL
- ELISA Plate Reader with filter (450 nm)
- Water bath (adjustable to 60 °C (140 °F)
- 15-30 mL recipients for the extraction
- Centrifuge
- Distilled water
- Stomacher, Mill, Mortar, Blender, etc
- Vortex mixer

15. Optional materials/equipment

- Homogenizer for sample extraction.
- The use of a repeating pipette minimizes the assay drift.
- An ELISA plate washer system reduces the washing time and improves consistency.
- Use fully automated ELISA analyzers (ELISA robots) for more convenience.

16. Reagent preparation

It is advisable to prepare reagents immediately before use and limited to the amount necessary for the number of samples plus the 6 standards, each in duplicates. Please note that all reagents must be at room temperature (15 – 25 °C/59 - 77 °F) at the time of use.

Preparation of the Washing Buffer.
Dilute 1:10 with distilled water; warm-up for 15 minutes at 37 °C (99 °F), if precipitated.

ELISA plate
Cut the foil bag beyond the zip. Take out only the number of strips required for the test to be executed (samples plus the 6 standards, both in duplicates) and put them onto the frame. Wells not required must be kept together with the drying agent in the foil bag, well-sealed, and stored at 2 – 8 °C (36 - 46 °F).

17. Sample and standard preparation

a) Wine samples:

1. Mix 0.5 mL of the wine sample with 2 mL of ddH_2O
2. Dilute 200 µL of the mixture with 800 µL of sample diluent.
3. Add 25 µL of reaction solution to 500 µL of the diluted wine sample.
4. Mix thoroughly and incubate for 1 min.
5. Add 100 µL of neutralizing solution to the activated sample.
6. Mix thoroughly and incubate for 1 min.
7. The derivatized sample is ready to be tested.

b) Fish samples:

1. Homogenize 10 g of fish sample with 100 mL ddH_2O in a mixer
2. Incubate for 30 minutes at 4°C (39 °F). *This step can be skipped if step 3 is performed with a centrifuge at 4°C (39 °F)*.
3. Centrifuge at a minimum of 3000 x g for at least 10 minutes.
4. Discard the upper fat layer and dilute 100 µL of the supernatant with 900 µL of sample diluent.
5. Add 25 µL of reaction solution to 500 µL of the diluted fish sample.
6. Mix thoroughly and incubate for 1 min.
7. Add 100 µL of neutralizing solution to the activated sample.
8. Mix thoroughly and incubate for 1 min.
9. The derivatized sample is ready to be tested.

c) Standard preparation:

Standards must be derivatized as follows before applying to the test:

1. Add 25 µL of reaction solution to 500 µL of each standard.
2. Mix thoroughly and incubate for 1 min.
3. Add 100 µL of neutralizing solution to the activated standard.
4. Mix thoroughly and incubate for 1 min.
5. The derivatized standards are ready to be used in the assay.

18. Test procedure

1. Prepare samples and standards as described above.
2. Dispense 100 µL derivatized standards or prepared samples in duplicate into the appropriate wells of the microtiter plate.
3. Add 50 µL of anti-histamine antibody into each well.
4. Incubate for 5 minutes at room temperature (15 – 25 °C/59 - 77 °F).
5. Wash the plate three times as follows: Discard the contents of the wells (dump or aspirate). Dispense 300 µL of diluted washing solution into each well. After the third repetition empty the wells again and remove residual liquid by striking the plate against a paper towel. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbances.
6. Dispense 100 µL of conjugate (anti-rabbit-IgG-HRP) into each well.
7. Incubate for 5 minutes at room temperature (15 – 25 °C/59 - 77 °F).
8. Wash the plate as described in step 5.
9. Dispense 100 µL of substrate solution into each well.
10. Allow the reaction to develop in the dark (the chromogen is light sensitive) for 5 minutes at room temperature (15 – 25 °C/59 - 77 °F).
11. Stop enzyme reaction by adding 100 µL of stop solution (0.5 M H_2SO_4) into each well. The blue color will turn yellow upon addition.
12. Wait for 1 minute. Read plate at 450nm (reference wavelength 620 nm) by using the plate reader and record OD values. The color is stable for about 30 minutes.

19. Calculation of the results

The standards are prepared for a direct determination of fish sample concentrations. The dilution of samples in the extraction process as described in the above stated sample preparation procedure is already considered. Additional dilution due to high sample concentration must be accounted for.

1. Calculate the average optical density (OD 450 nm) for each set of reference standards and samples.
2. Construct a standard curve by plotting the mean optical density obtained for each reference standard (x-axis) against its concentration (y-axis). Alternatively, the evaluation can be carried out by software. In this case the 4-parameter method is preferred.
3. Using the mean optical density (OD) value for each sample, determine the corresponding concentration of histamine in ppm from the standard curve.
4. Due to a deviating sample preparation process the results for wine samples must be additionally multiplied by 0.25 in order to obtain the concentration of histamine in the original sample.

Assay data and acceptance/rejection criteria of measured values

The Lot-specific data and acceptance/rejection criteria can be found in the Certificate of Analysis included in each kit.

Example assay layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	S0	S0	SP3	SP3	etc.	etc.	etc.	etc.	etc.	etc.	etc.	etc.
B	S1	S1	SP4	SP4	etc.	etc.	etc.	etc.	etc.	etc.	etc.	etc.
C	S2	S2	etc.	etc.	etc.	etc.	etc.	etc.	etc.	etc.	etc.	etc.
D	S3	S3	etc.	etc.	etc.	etc.	etc.	etc.	etc.	etc.	etc.	etc.
E	S4	S4	etc.	etc.	etc.	etc.	etc.	etc.	etc.	etc.	etc.	etc.
F	S5	S5	etc.	etc.	etc.	etc.	etc.	etc.	etc.	etc.	etc.	etc.
G	SP1	SP1	etc.	etc.	etc.	etc.	etc.	etc.	etc.	etc.	etc.	etc.
H	SP2	SP2	etc.	etc.	etc.	etc.	etc.	etc.	etc.	etc.	etc.	etc.

S0: Zero-Standard (without antigen); S1-5: Standards; SP: Samples

20. Performance indications

Intra-Plate-Variance (well-to-well): 6 %

Inter-Plate-Variance (plate-to-plate): 7 %

(Values can vary from batch to batch)

21. Disclaimer

These products are made from high quality raw materials. No warranty of any kind is made either expressed or implied, as to their suitability other than to measure the target antigen content when used exactly in accordance with these instructions, except regarding the quality of this materials.

Use of the kit for any other purpose is outside its intended use. Any damages, including consequential or special damage or expense arising directly or indirectly from using this product, are limited to the replacement value of the kit.

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