Test Instruction

Histamine Rapid

96 Tests

Enzyme Immunoassay
for the Quantitative
Determination of
Histamine in Fish and Wine

Cat.-No.: HIS-E03

Version: December 1st, 2022

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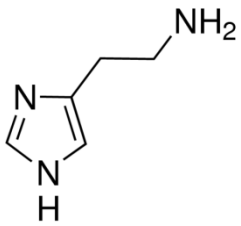
Sensitivity	0.3 – 0.7 mg/kg (ppm)
Recovery	84-102%
Incubation Time	17 min incl. derivatization



General Information

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 allergical reactions appear.
But histamine can also enter the human body via the nutrition and can thus cause pseudoallergic food intolerances. Food intolerances, which are caused by increased histamine concentrations, are clinically characterized by rash, diarrhoea, vomiting, nausea, itching, headache and asthma. The extent of the reaction is dependent on the ingested amount of histamine.
Separated from histamine intolerances toxic reactions exist, which are caused by very high histamine concentrations. 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 1019/2013 in the European Union the limit for histamine in fish or fish products is 100 ppm. Thus a monitoring of fish and fish products with respect to the concentration of histamine is obligatory.

The **Gold Standard Diagnostics Histamine RAPID ELISA** represents a sensitive detection system and is particulary capable of the rapid quantification of histamine concentrations in fish and wine.

Principle of the Test

The **Gold Standard Diagnostics Histamine** quantitative test is based on the principle of the enzyme linked immunosorbent assay. 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 given 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 given 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 colour. The colour development is inhibited by the addition of a stop solution, and the colour turns yellow. The yellow colour is measured photometrically at 450 nm. The concentration of histamine is indirectly proportional to the colour intensity of the test sample.

Precautions

Full compliance of the following good laboratory practices (GLP) will determine the reliability of the results:

1. Prior to beginning the assay procedure, bring all reagents to room temperature (20-25°C).

2. All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.

3. Once the assay has been started, all subsequent steps should be completed without interruption and within the recommended time limits.

4. Replace caps in all the reagents immediately after use. Do not interchange vial stoppers.

5. Use a separate disposable tip for each specimen to prevent cross-contamination.

6. All specimens and standards should be run at the same time, so that all conditions of testing are the same.

7. Do not mix components from different batches.

8. Do not use reagents after expiration date.

9. Check both precision and accuracy of the laboratory equipment used during the procedure (micropipets, ELISA reader etc.).

Health and safety instructions

1. Do not smoke or eat or drink or pipet by mouth in the laboratory.

2. Avoid contact of substrate and stop solution with skin and mucosa (possible irritation, burn or toxicity hazard). In case of contact, rinse the affected zone with plenty of water.

3. Handling and disposal of chemical products must be done according to good laboratory practices (GLP).

4. The reaction solution contains 1,4-benzoquinone. If a skin contact occurs, the afflicted area must be rinsed with plenty of water.

Reagents

The kit contains reagents for 96 determinations. They have to be stored at 2-8°C. Expiry data are found on the labels of the bottles and the outer package.

1. Microtiter plate consisting of 12 strips with 8 breakable wells each, coated with histamine conjugate.

2. Histamine Standards (0; 2; 6; 12; 24; 72 ppm): 6 vials with 4.0 mL each. Before application in the test the standards have to be derivatized (see *Reagent Preparation* section).

3. Reaction Solution: 3 mL, dyed brown, ready-to-use.

4. Neutralizing Solution: 15 mL, ready-to-use.

5. Anti-Histamine Antibody (rabbit): 6 mL, dyed blue, ready-to-use.

6. Conjugate (anti-rabbit-IgG-HRP): 15 mL, dyed red, ready-to-use.

7. Substrate Solution (TMB): 15 mL, ready-to-use.

8. Stop Solution (0.5 M H_2SO_4): 15 mL, ready-to-use.

9. Sample Diluent (PBS): 2 x 60 mL, ready-to-use.

10. Washing Solution (PBS + Tween 20): 60 mL as 10x concentrate, dyed blue. Dilute 1+9 with distilled water. If during the cold storage crystals precipitate, the concentrate should be warmed up at 37°C for 15 minutes.

11. Plastic bag to store unused microtiter strips.

12. Instruction Manual.

Additional Instrumentation and Reagents (not provided)

Instrumentation

- 25, 50, 100 and 500 µL micropipets
- Microtiter plate shaker
- ELISA reader (450 nm)
- Centrifuge
- Ultra-Turrax, mixer, vortex

Reagents

- Double distilled water

Sample Preparation

Wine

- Mix 500 µL wine with 2 mL of double distilled water.
- Dilute 200 µL of the mixture with 800 µL of sample diluent.
- Add 25 µL of reaction solution to 500 µL of the diluted wine sample.
- Mix thoroughly and incubate for 1 min.
- Add 100 µL of neutralizing solution to the activated sample.
- Mix thoroughly and incubate for 1 min.
- Test the derivatized sample in the ELISA

Fish

- Homogenize 10 g of fish with 100 mL double distilled water in a mixer or with a turrax.
- Incubate for 30 minutes at 4°C. Alternatively this step can be skipped and a centrifuge cooled down to 4°C has to be used in the next step.
- Centrifuge at a minimum of 3000 g for at least 10 minutes.
- Discard the upper fat layer and dilute 100 µL of the supernatant with 900 µL of sample diluent.
- Add 25 µL of reaction solution to 500 µL of the diluted fish sample.
- Mix thoroughly and incubate for 1 min.
- Add 100 µL of neutralizing solution to the activated sample.
- Mix thoroughly and incubate for 1 min.
- Test the derivatized sample in the ELISA

Reagent Preparation

Before application in the test the standards have to be derivatized as follows:

- Add 25 µL of reaction solution to 500 µL of each standard.
- Mix thoroughly and incubate for 1 min.
- Add 100 µL of neutralizing solution to the activated sample.
- Mix thoroughly and incubate for 1 min.
- Apply the derivatized standards in the ELISA

Procedure

1. Prepare samples and standards as described above.

2. Pipet 100 µL derivatized standards or prepared samples in duplicate into the appropriate wells of the microtiter plate.

3. Add 50 µL of histamine antibody into each well.

4. Incubate for 5 minutes at room temperature.

5. Wash the plate three times as follows: Discard the contents of the wells (dump or aspirate). Pipet 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 absorbencies.

6. Pipet 100 µL of conjugate (anti-rabbit-IgG-HRP) into each well.

7. Incubate for 5 minutes at room temperature.

8. Wash the plate as outlined in step 5.

9. Pipet 100 µL of substrate solution into each well.

10. Allow the reaction to develop in the dark (e.g. cupboard or drawer; the chromogen is light-sensitive) for 5 minutes at room temperature.

11. Stop enzyme reaction by adding 100 µL of stop solution (0.5 M H_2SO_4) into each well. The blue colour will turn yellow upon addition.

12. After thorough mixing, measure absorbance at 450 nm (reference wavelength 620 nm), using an ELISA reader. The colour is stable for 30 minutes.

Calculation of 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 has to be accounted for.

1. Calculate the average optical density (OD 450 nm) for each set of reference standards or samples.

2. Construct a standard curve by plotting the mean optical density obtained for each reference standard against its concentration in ppm on semi-log graph paper with the optical density on the vertical (y) axis and the concentration on the horizontal (x) axis. Alternatively the evaluation can be carried out by software. In this case the 4-parameter method should be preferred.

3. Using the mean optical density (OD) value for each sample, determine the corresponding concentration of histamine in ppm from the standard

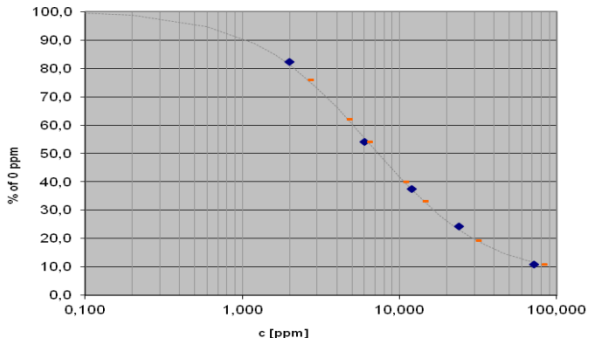
curve. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed.

4. Due to a deviating sample preparation process the results for **wine samples additionally have to be multiplied with 0.25** in order to get the real concentration of the sample.

Typical Standard Values

The following table contains an example for a typical standard curve. The binding is calculated as percent of the absorption of the 0 ppm standard. These values are only an example and should not be used instead of the standard curve which has to be measured in every new test.

Histamine (ppm)	(% binding of 0 ppm)
0	100
2	82
6	54
12	37
24	24
72	11



Performance

Sensitivity

The limit of detection (LOD) of the **Gold Standard Diagnostics Histamin Rapid test** is 7 ppb for the standard curve.

The limit of quantification (LOQ) of the **Gold Standard Diagnostics Histamin Rapid test** is 24 ppb for the standard curve.

Validation experiments with fish matrices and wine resulted in the following LODs and LOQs [ppm].

Matrix	LOD	LOQ
Codfish	0.4	2.0
Plaice	0.4	1.7
Salmon	1.1	2.5
Trout	0.7	2.6
Tuna	0.5	1.5
Wine	0.3	0.4

Recovery

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

Linearity

The serial dilution of spiked samples (fish, wine) resulted in a dilution linearity of 92-109%.

Precision

Intra-assay Precision	6%
Inter-assay Precision	7%

Cross-reactivity relative to histamine (=100%)

N-Acetylhistamine	0.02%
1-Methylhistamine	5%
Histidine	0%
Serotonin	0%

References

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