



## Test Instruction

Histamine

96 Tests

Enzyme Immunoassay  
for the Quantitative  
Determination of  
Histamine in Food

Cat.-No.: HIS-E02

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Sensitivity	2 ng/mL
Recovery (wine, fish, cheese)	90%
Incubation Time	80 min

### 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, endogenous 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 the nutrition and can thus cause pseudoallergic food intolerances. The appearing symptoms are not distinguishable from those of a real allergy. The term 'food allergy' is however only used, if the pathologic symptoms develop in the course of an immunological reaction. Non-immunological reactions to food are defined as 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. These reactions can be enhanced by a lack of diaminoxidase.

Separated from histamine intolerances toxic reactions exist, which are caused by very high histamine concentrations. Toxic histamine concentrations, which can cause the so-called scombroid reaction, appear after bacterial degradation of protein-rich food, especially fish.

Histamine is found above all in cheese, fish, spinach, smoked products and red wine. The histamine concentrations in different types of food, but also in the same food show frequently great differences. In different types of cheese there can e.g. be found histamine contents between 600 and 1000 mg/kg.

The **Gold Standard Diagnostics Histamine ELISA** is a quick, economical and sensitive method to detect histamine in wine, fish or cheese. After an appropriate sample preparation, 40 samples can be tested in duplicate within 80 minutes.

### 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 30 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 30 minutes incubation, the plate is

washed again. Then a substrate solution is added and incubated for 20 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. Wear disposable gloves whenever handling patient specimens.
3. 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.
4. Handling and disposal of chemical products must be done according to good laboratory practices (GLP).

5. 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; 4; 10; 40; 100 ng/mL): 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, ready-to-use.
4. Neutralizing Solution: 15 mL, ready-to-use.
5. Anti-Histamine Antibody (rabbit): 6 mL, dyed red, 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<sub>2</sub>SO<sub>4</sub>): 15 mL, ready-to-use.
9. Sample Diluent (PBS): 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. Two plastic foils to cover the strips during the incubation.
12. Plastic bag to store unused microtiter strips.
13. Instruction Manual.

## Additional Instrumentation and Reagents (not provided)

### Instrumentation

- 25, 50, 100 and 500 µL micropipets
- Microtiter plate shaker
- ELISA reader (450 nm)
- Mixer
- Centrifuge

## Reagents

- 0.1 M Hydrochloric acid (HCl)

## Sample Preparation

### Wine

- 500  $\mu\text{L}$  wine are mixed with 500  $\mu\text{L}$  sample diluent (1:2).
- 50  $\mu\text{L}$  reaction solution are pipetted to this diluted wine sample, mixed properly and incubated for 20 minutes at room temperature in the dark.
- 200  $\mu\text{L}$  neutralizing solution is pipetted to the sample, mixed properly, and incubated for 20 minutes at room temperature.
- The sample can now be directly inserted into the test.

### Cheese and Fish

- 10 g cheese or fish are homogenised with 50 mL 0.1 M HCl in a mixer for 5 minutes.
- The homogenous sample is further centrifuged for 10 minutes at 3000 g. Three phases develop: an upper solid fatty layer, a middle turbid aqueous layer and a pellet at the bottom.
- 500  $\mu\text{L}$  of the turbid aqueous phase are removed. Care has to be taken, that no fat particles of the upper solid phase are pipetted. If this happens, the fat must be taken away and discarded. The resulting 500  $\mu\text{L}$  of sample are mixed with 500  $\mu\text{L}$  of sample diluent (1:2).
- To this diluted cheese or fish sample 50  $\mu\text{L}$  reaction solution are pipetted, mixed thoroughly and incubated for 20 minutes at room temperature in the dark.
- 200  $\mu\text{L}$  neutralizing solution is pipetted to the sample, mixed properly, and incubated for 20 minutes at room temperature.
- The sample can now be directly inserted into the test.

Note: The differently high concentrations of histamine in the various food samples can require, that the samples have to be extra diluted 1:10 or 1:100 with sample diluent.

## Reagent Preparation

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

- To 500  $\mu\text{L}$  of each standard 25  $\mu\text{L}$  reaction solution is pipetted, mixed thoroughly and incubated for 20 minutes at room temperature in the dark.
- 100  $\mu\text{L}$  neutralizing solution is pipetted to the respective standards, mixed thoroughly and incubated for 20 minutes at room temperature.
- The standards can now be inserted into the test directly.

## Procedure

1. Prepare samples as described above.
2. Pipet 100  $\mu\text{L}$  derivatized standards or prepared samples in duplicate into the appropriate wells of the microtiter plate. Immediately add 50  $\mu\text{L}$  histamine antibody into each well.
3. Cover the microtiter plate with a plastic foil and incubate for 30 minutes at room temperature on a microtiter plate shaker (or 45 minutes without shaker).
4. Wash the plate three times as follows: Discard the contents of the wells (dump or aspirate). Pipet 300  $\mu\text{L}$  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.
5. Pipet 100  $\mu\text{L}$  of conjugate (anti-mouse-IgG-HRP) into each well.
6. Cover the microtiter plate with a plastic foil and incubate for 30 minutes at room temperature on a microtiter plate shaker (or 45 minutes without shaker).
7. Wash the plate as outlined in 4.
8. Pipet 100  $\mu\text{L}$  of substrate solution into each well.
9. Allow the reaction to develop in the dark (e.g. cupboard or drawer; the chromogen is light-sensitive) for 20 minutes at room temperature.
10. Stop enzyme reaction by adding 100  $\mu\text{L}$  of stop solution (0.5 M  $\text{H}_2\text{SO}_4$ ) into each well. The blue colour will turn yellow upon addition.
11. 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

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 ng/mL on semi-log graph paper with the optical density on the vertical (y) axis and the concentration on the horizontal (x) axis.

- Using the mean optical density value for each sample, determine the corresponding concentration of histamine in ng/mL from the standard curve. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed.
- The diluted samples must be further converted by the appropriate dilution factor. The dilution factor is **2 for wine** and **10 for cheese and fish** extraction according to the sample preparation procedure as described above.
- Bieger, W.P. und von Baehr, R.: *Nahrungsmittelallergien I. Allgemeine Grundlagen*, Naturheilpraxis, 49 (3), 313 - 326 (1996).
- Kaiser, R. und Bauer, Ch.: *Vorkommen und Bestimmung von Histamin in Käse*, Lebensmittelchemie, 49, 84 (1993).
- Wantke, F.M. et al.: *The red wine provocation test: intolerance to histamine as model for food allergy*, Allergy Proc. 15, 27 - 32 (1994).
- Wüthrich, B.: *Lebensmittelallergien und -intoleranzen*, Lebensmittelchemie, 50, 155 - 156 (1996).

### 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 ng/mL 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 (ng/mL)	(% binding of 0 ng/mL)
0	100
2	65
4	49
10	34
40	18
100	13

### Performance

#### *Sensitivity*

The sensitivity of the **Gold Standard Diagnostics Histamine ELISA** is 2 ng/mL (based on the standard curve).

#### *Recovery*

The recovery of spiked samples was determined to 90% for wine, fish or cheese samples.

#### *Intra-assay Precision*

The intra-assay variation of the histamine test was determined to 5%.

#### *Cross-reactivity relative to histamine (=100%)*

N-Acetylhistamine	6%
1-Methylhistamine	0.05%
Histidine	0%
Serotonin	0%

### References

- Götz, M. et al: *Histaminintoleranz und Diaminoxidasemangel*, Allergologie, 19 (9), 394 - 398 (1996).