Kit for the quantitative analysis of histamine

Histamine Test

For 60 assays
Code 61341

User's manual
(New protocol)

Histamine Test is a colorimetric enzymatic assay for the quantitative analysis of histamine in, fresh or frozen fish and canned tuna (thick broth may affect the test results).

- Easy extraction: Do not need any purification to remove substances that interfere with the assay, which is needed in HPLC method or AOAC method.
- Easy procedure and short assay time: Complicated procedure is unnecessary, which is needed in HPLC method or EIA methods.

Principle of Measurement

Histamine Test is a colorimetric enzymatic assay for the quantitative analysis of histamine. Histamine dehydrogenase catalyses the oxidation of histamine.

This reaction in the presence of 1-methoxy-5-methylphenazinium methylsulfate (1-methoxy PMS) can produce colored tetrazolium salt that can be measured around 470 nm.

Product Specifications

1. No interference is seen with other amines, such as putrescine and cadaverine.
2. Quantitative range: 10ppm – 150ppm of histamine in sample (0.4ppm – 6ppm in sample solution) in case using a spectrophotometer which optical path length is 2 cm, such as Absorptiometer B (ref. "Recommended Instruments"). In case using a spectrophotometer which optical path length is 1 cm, the quantitative range is 20ppm – 300ppm (0.8ppm－12ppm in sample solution).
3. Testing time: 20 minutes once sample solutions have been prepared and reagents have been dispensed to test tubes.

Composition of Kit

1. Enzyme reagent:
   6 green -labeled vials
   These contain histamine dehydrogenase.
2. Colorimetric reagent:
   6 magenta-labeled vials
   These contain tetrazolium salt (WST-8; 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-di sulphonyl)-*2H*-tetrazolium, monosodium salt) and 1-methoxy PMS.
3. Buffer:
   24 ml x 3 pink-labeled vials
   These contain Tris-HCl buffer.
4. Histamine Standard solution:
   30 ml x 1 blue-labeled bottle
   This contains histamine.

Note: The kit contains reagents for 60 assays including measurement of the standard. The one-point standard calibration curve method is used for this kit. Therefore not only sample but also standard is needed to measure in each analysis. In case of one sample, the following two sets of measurements are required: (a) a sample and a sample blank, and (b) a standard solution and a reagent blank. In case of the assays for multiple samples, only one set of (b) is required for the multiple samples. Therefore, if the whole kit is used at one time, up to 59 samples can be measured. Meanwhile, up to 50 samples can be measured if the kit is used in 10 times.

Precautions

1. Do not use an expired kit. (Expiration date is printed on the kit box)
2. Glassware should not be used for extraction and measurement purposes, because histamine may adhere to glass. Using glassware may affect test results.
3. Do not mix reagents from one kit serial with reagents from a different kit serial.
4. Kit should be brought to 18–30°C prior to use.
5. Avoid prolonged storage of kits at ambient temperatures.
6. While preparing the sample solution, carry out quickly to avoid the contamination of microorganisms. To avoid decay of the sample, cool the sample (0–10°C) during homogenization and a series of operations after boiling extraction.
7. During boiling step of the sample, be careful for sudden boiling of the sample and scalding oneself.
8. Change the filter paper if it takes more than 5 minutes to filter the sample. If the sample contains a lot of fat, cool the extracted sample solution enough to separate solid phase and liquid phase before filtration.
9. Freeze the samples if you do not assay them immediately. Freezing and thawing are recommended to limit only once. While thawing the samples, keep them below 10°C. Be careful for the contamination of microorganisms during thawing the samples.
10. Accurate incubation time is required. Otherwise you may not get precise result.
11. Close the cap of the histamine standard solution immediately to avoid evaporation.

Recommended Instruments

Absorptiometer B (model ABS-B470)
(supplier: Kyoritsu Chemical-Check Lab., Corp.),
or a spectrophotometer which is capable to measure at around 470 nm.

*Use a cuvette which is suitable for 1.5ml or less sample solution.

Materials Required but Not Provided

(1) Homogenizer
(2) Scale (which is able to weigh 1 g) and medical spoon
(3) Heat-resistant plastic test tube with cap, which can close tightly (50 ml conical tube, for extraction)
(4) Pipette (to measure 0.5 ml) and tips
(5) Spatula
(6) Distilled water
(7) Filters paper (No. 5C) and funnels (or centrifuge)
(8) Small plastic test tubes (About 10 ml, for dilution of histamine standard solution and colorimetric assay reaction) and test tube racks
(9) Incubator (37°C)
(10) Heater (ex. gas stove) and pot
(11) Sample treatment buffer:
0.1 M EDTA·2Na (pH 8.0) solution
(12) Ice

Note: Preparation of the sample treatment buffer:
Weigh 37.2 g of EDTA·2Na·2H_2O and dissolve in about 750 ml of distilled water. Adjust its pH to pH8.0 using sodium hydroxide solution or potassium hydroxide solution. Adjust its volume to 1,000 ml with distilled water. It can be stored in a clean vessel with cover at room temperature.

Or the sample treatment buffer can be prepared by five times dilution of a commercial 0.5 M EDTA (pH 8.0) solution.

Instructions for Use

1. Extraction and Preparation of the Sample Solutions

(1) Weigh about 10 grams of the fish tissue and homogenize. Weigh out precisely 1 g of the homogenized sample and transfer to a heat- resistant test tube with cap.
(2) Add precisely 24 ml of sample treatment buffer. Sample is diluted 25 fold by above operations.
(3) Cover the cap tightly and suspend the sample well. Put the test tube at a tube stand. Boil it for 20 minutes.
(4) Cool the tube by placing it on ice (until it becomes < 20ºC).
(5) Suspend the sample well using clean spatula and cool it again on ice bath to separate solid phase and liquid phase. Solid phase includes fat.
(6) Filter the contents through folded filter paper (No. 5C) into a clean plastic tube. Or you can centrifuge (10,000 x g, 5 min) and collect the supernatant.
(7) The sample solution is now ready to assay.

2. Preparation of the Reagent

(1) Colorimetric reagent:
Colorimetric reagent is kept under vacuum in magenta-labeled vial. Add exactly 11 ml of distilled water to the vial. Stir the vial gently so as not to produce foam until the content is completely dissolved. Protect from light and keep it between 0ºC and 10 ºC before you use. Since the colorimetric reagent is extremely sensitive against the natural sunlight, handle the reagent in some place protected from the natural sunlight by some kind of window shade. One vial of colorimetric reagent can be used for 10 assays under normal condition.

We recommend using up the reagent at one time. But if not possible, you may keep it between 2 ºC and 8 ºC up to one week or keep it at -10 ºC or below up to one month. Freezing and thawing are recommended to limit up to three times. When thawing the reagent, use running water and thaw it as quickly as possible the samples, and then keep it below 10°C.

(2) Enzyme reagent:
Enzyme reagent is kept under vacuum in green -labeled vial. Pull the rubber plug very slowly not to fly loss the reagent powder. Add exactly 6 ml of the buffer (pink-labeled vial) to the green-labeled vial. Stir the vial gently so as not to produce foam until the content is completely dissolved. Keep it between 0ºC and 10 ºC before you use. One vial of enzyme reagent can be used for 10 assays under normal condition.

We recommend using up the reagent at one time. But if not possible, you may keep it at -10 ºC or below up to one month. Freezing and thawing are recommended to limit up to three times. When thawing the reagent, use running water and thaw it as quickly as possible the samples, and then keep it below 10°C.

3. Assay Procedure

Refer to the table "Reagent combination" below. Incubate all the assay tubes simultaneously and avoid sunlight.

(1) To set the absorbance of the spectrophotometer to zero, distilled water should be used as reference according to its instruction manual.
(2) To assay N samples, prepare (2N+2) plastic test tubes.
(3) To carry out sample assay, add 0.5 ml of the extracted sample solution. Then add 0.5 ml each of the colorimetric reagent and the enzyme solution. Mix well and incubate at 37ºC for 15 min. Do not

irradiate strong light, especially sunlight during a series of operations. Possible protection from light is desirable.

After the incubation, measure the absorbance at around 470 nm (Es value). If the Es value is larger than 1.0, dilute the extracted sample solution with distilled water and perform the assay again.

(4) To carry out sample blank assay, add 0.5 ml of the buffer instead of the enzyme solution. Carry on the same operation as in (3). Measure the absorbance at around 470 nm (Eb value).

(5) To carry out histamine standard assay, use 0.5 ml of the histamine standard solution instead of the extracted sample solution. Carry on the same operation as in (3). Measure the absorbance at around 470 nm (Estd value). The Estd value should be around 0.85 in case using a spectrophotometer which optical path length is 2 cm or around 0.5 in case using a spectrophotometer which optical path length is 1 cm under normal conditions. If not, check the operation procedure and perform the assay again.

(6) To carry out reagent blank assay, add 0.5 ml of distilled water instead of the extracted sample solution and 0.5 ml of the buffer instead of the enzyme solution. Carry on the same operation as in (3). Measure the absorbance around 470 nm (Ec value). The Ec value should be less than 0.10 in case using a spectrophotometer which optical path length is 2 cm or less than 0.05 in case using a spectrophotometer which optical path length is 1 cm under normal conditions. If not, check the operation procedure and perform the assay again.

Absorbance measurement conditions

Wavelength: around 470 nm
Reference to set the absorbance to zero: distilled water
Final volume: 1.5 ml

Table. Reagent combination (ml)

	Absorbance of the sample	Absorbance of sample blank	Absorbance of standard solution	Absorbance of reagent blank
Extracted sample solution	0.5	0.5	–	–
Histamine standard solution	–	–	0.5	–
Distilled water	–	–	–	0.5
Colorimetric Reagent	0.5	0.5	0.5	0.5
Enzyme solution	0.5	–	0.5	–
Buffer	–	0.5	–	0.5
	Es	Eb	Estd	Ec

4. Interpretation of Results

You can determine the histamine concentration of the fish sample by the following calculation:

The histamine concentration (mg/L = ppm)
= (Es – Eb) ÷ (Estd – Ec) × 4 × 25× df
= (Es – Eb) ÷ (Estd – Ec) × 100 × df

Es: Absorbance of the sample, Eb: Absorbance of the sample blank, Estd: Absorbance of the standard solution, Ec: Absorbance of the reagent blank, df: dilution factor of the sample solution. (If the extracted sample solution wasn't diluted for the Assay Procedure (3), df=1.) Figures "4" and "25" in the formula mean that the histamine concentration of the standard solution is 4 ppm, and that sample has been diluted 25 fold by extraction procedure, respectively.

Protocol for Spike and Recovery Tests

This kit is basically designed for measuring histamine in raw fish. Histamine in seasoned foods and fermented foods might not be properly measured, although histamine in some fish products, such as canned oiled fish and canned boiled fish, would be measurable. Therefore, following spike and recovery tests would be required for food stuff other than raw fish for assessing suitability of this kit.

Note: Not all reagents and equipments required in this section are described in "Instruction for Use".

1. Preparation of Histamine Solution (1000ppm) for Spike Test

Histamine dihydrochloride was dried in desiccators (non-heater) for two hours. Then weigh 0.167 g of the dried histamine dihydrochloride, dissolve it in 0.1N HCl , and adjust to 100 mL.

2. Preparation of Sample Solution

(1) Weigh about 10 grams of the food stuff and homogenize.

(2) Prepare two heat-resistant conical tubes with caps (tubes A and B). Weigh out precisely 1 g of the homogenized sample and transfer to the tube A. Then weigh out precisely 1 g of the homogenized sample and transfer to the tube B.

(3) Add 0.1 ml of the histamine solution (1000ppm) to the tube A. Add 0.1 ml of distilled water to the tube B.

(4) Add precisely 24 ml of sample treatment buffer to tube A. Then add precisely 24 ml of sample treatment buffer to tube B. Cover both tubes with caps tightly and suspend the sample well. Put the tubes at a tube stand. Boil it for 20 minutes. (Samples are diluted 25 fold by above operations.)

(5) Cool the tubes by placing it on ice.

(6) Suspend the sample well using clean spatula.

(7) Cool it again on ice bath to separate solid phase and liquid phase.

(8) Filter the contents of (7) through folded filter paper

(No. 5C) into clean plastic tubes A and B, respectively. Or you can centrifuge (10,000 x g, 5 min) and collect the supernatant to clean tubes A and B, respectively. Each of tubes is referred to as sample solution A and sample solution B, respectively.

3. Measurement

Measure histamine concentrations in the sample solution A and the sample solution B according to "Instructions for Use 3. Assay Procedure and 4. Interpretation of Results." Triplicate measurements and calculating mean value are recommended.

4. Calculation of Recovery Rate

You can determine the recovery rate from the sample by the following calculation:

Recovery rate (%) = (Conc. A – Conc. B) ÷100ppm x 100%

Con. A: Histamine concentration (ppm) in the sample solution A, Conc. B: Histamine concentration (ppm) in the sample solution B, 100ppm: Histamine concentration spiked in tube A.

Disposal Methods

The vessels of the colorimetric reagent and the enzyme are consisted of glass, rubber, aluminum. The vessels of the buffer and the histamine standard solution are consisted of polyethylene and polypropylene cap. It would be better to separate the parts and dispose of each one properly in accordance with the local regulations outlined by the local governments for proper disposal of waste materials.

For Safety Use

Pay attention to the points listed below for safe operation of this kit.

1. Histamine Test is not recommended or intended for the diagnosis of disease in humans or animals.
2. This kit is designed for a voluntary testing. As necessary, use an official method together with this kit
3. This kit is designed for use by quality control personnel and others familiar with histamine analysis in fish.
4. Don't swallow or contact the reagents supplied with this kit with skin or eyes. In case of swallowing or contact with the skin or eyes, rinse immediately with plenty of water and seek medical advice. Histamine may cause allergic-like reaction to human body.
5. Wear protection gloves when washing equipments after use.
6. Store and discard this kit with care so that you do not contaminate food or other products with the reagents and materials supplied with the kit.
7. Don't mix the reagents of this kit with other chemicals. Some toxic fumes might be generated.
8. Please follow the "Instructions for Use". Not to attempt using any reagents in either different dilution ratio or different combination ratio from the instruction.
9. Keep this kit away from children and infants.

Storage of Kit

Store the kit at 2–8°C in a refrigerator. DO NOT FREEZE.

Warranty

Kikkoman Biochemifa Company makes no warranty of any kind, either expressed or implied, except that the materials from which its products are made are standard quality. If any materials are defective, Kikkoman Biochemifa Company will provide a replacement product. Buyer assumes all risk and liability resulting from the use of this product. There is no warranty of merchantability for this product, or for the fitness of the product for any purpose. Kikkoman Biochemifa Company shall not be liable for any damages, including special or consequential damages, or expenses arising directly or indirectly from the use of this product.

Manufacturer

Kikkoman Biochemifa Company

2-1-1, Nishi-shinbashi, Minato-ku, Tokyo 105-0003, Japan
Tel: +81 3 5521 5492
Fax: +81 3 5521 5498
E-mail: biochemifa@mail.kikkoman.co.jp
URL: http://biochemifa.kikkoman.co.jp/e/