Kit for the quantitative analysis of histamine

Histamine Test

For 60 assays
Code 61341

User's manual for raw or canned fish

Histamine Test is a colorimetric enzymatic assay for the quantitative analysis of histamine in fresh, frozen and canned Scombridae fish species such as tuna, bonito and mackerel.

- The kit has been validated and approved for AOAC-RI PTM certification (License Number:041802).
- Easy extraction provides an advantage over HPLC methods or the AOAC official Method 977.13.
- The ease of use and short testing time provide advantages over HPLC methods or EIA methods.

Intended Use

The method is intended for the determination of histamine in fresh or frozen raw Scombridae fish species such as tuna, bonito and mackerel, and canned these fish in water or oil.

Intended User

The test kit is designed for use by personnel with basic laboratory skills in a laboratory environment.

Principle of Measurement

Histamine dehydrogenase catalyzes the oxidation of histamine. This reaction in the presence of 1-methoxy-5-methylphenazinium methylsulfate (1-methoxy PMS) and 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulphonyl)-*2H*-tetrazolium, monosodium salt (WST-8) produces a colored tetrazolium salt that is measured at 470 nm.

Product Specifications

1. No interference is seen with most other amines. However, at high concentrations, agmatine causes a strong positive signal and putrescine causes a weak positive signal.
2. Quantitative range: Unit : mg/kg

	Optical path length of a spectrophotometer	
	1 cm	2 cm*
Raw or canned fish	20– 300	10 – 150
After 25-fold dilution with sample treatment buffer	0.8－12	0.4 – 6

* Absorptiometer B or Absorptiometer RGB are recommended (ref. “Recommended Instruments”).

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 1-methoxy PMS and WST-8.
3. Buffer: 24 mL × 3 pink-labeled vials
   These contain Tris-HCl buffer.
4. Histamine standard solution:
   30 mL × 1 blue-labeled bottle
   This contains 4 mg/kg histamine.

Note: The kit contains reagents for 60 assays including measurement of the standard. A one-point standard calibration curve method is used for this kit. Therefore, not only sample but also standard is must be measured in each analysis. In the 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 the case of multiple samples, only one set of (b) is required.

Precautions

1. Do not use an expired kit. (The 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 different kit lots.
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, do it quickly to avoid contamination by microorganisms. To avoid decay of the sample, cool the sample (0–10°C) after the homogenization and during a series of operations after boiling extraction.
7. During the boiling step of the sample, be careful of sudden boiling and avoid 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 the solid matter and the liquid matter before filtration.
9. Freeze the samples if you do not assay them immediately. Freezing and thawing are recommended to be done only once. While thawing the samples, keep them below 10°C. Be careful of contamination by microorganisms during thawing.
10. Accurate reaction time is required. Otherwise you may not get precise results.
11. Close the cap of the histamine standard solution immediately to avoid evaporation.
12. If the samples contain high levels of agmatine due to other fish species or fermentation process, false positive results may occur.

Recommended Instruments

Absorptiometer B (model ABS-B470) or Absorptiometer RGB (model DPM2-ABS) (Supplier:

Kyoritsu Chemical-Check Lab., Corp.), or a spectrophotometer capable of measurement at 470 nm.
*Use a cuvette suitable for ≤ 1.5 mL sample solution. Spectrophotometers with 1-cm or 2-cm optical path length are suitable.

Materials Required but Not Provided

(1) To prepare the histamine solution: histamine dihydrochloride, 0.1N HCl, plastic graduated cylinder (100 mL) and desiccator (without heat)
(2) Homogenizer
(3) Balances (capable of weighing 0.1 g and 1 mg)
(4) Heat-resistant Plastic conical tube (50 mL) with tight-fitting cap, for extraction
(5) Pipet (capable of measuring 0.5 and 0.1 mL) and tips
(6) Spatula
(7) Distilled water
(8) Filters paper (Advantec Grade No. 5C ashless filter paper or equivalent) and plastic funnels (or centrifuge)
(9) Graduated cylinder (25 mL)
(10) Small plastic test tubes (About 10 mL, for dilution of histamine standard solution and colorimetric assay reaction) and test tube racks
(11) Incubator (37 ± 1°C)
(12) Thermometer
(13) Timer
(14) Vortex mixer
(15) Boiling water bath
(16) Ice
(17) Sample treatment buffer: 0.1 M EDTA·2Na (pH 8.0) solution
(18) To prepare the sample treatment buffer: $EDTA{\cdot}2Na{\cdot}2H_2O$, NaOH or KOH solution, a beaker (1,000 mL), a graduated cylinder (1,000 mL), a clean vessel with cover and pH meter

Note: Preparation of the sample treatment buffer:
Weigh 37.2 g of $EDTA{\cdot}2Na{\cdot}2H_2O$ and dissolve in about 750 mL of distilled water. Adjust to pH 8.0 using NaOH or KOH solution. Adjust to 1,000 mL with distilled water using a 1,000 mL graduated cylinder. Store in a clean vessel with cover at room temperature up to 6 months.
Alternatively, the sample treatment buffer can be prepared by five-fold dilution of a commercial 0.5 M EDTA (pH 8.0) solution.

Instructions for Use

1. Extraction and Preparation of the Sample Solutions

(1) Homogenize at least 10 g of the fish tissue. Weigh out precisely 1.0 g of the homogenized sample and transfer to a heat-resistant test tube with cap.
(2) Add 24.0 mL of the sample treatment buffer with a 25 mL graduated cylinder to achieve a 25-fold dilution. (Sample is diluted 25 fold by these operations.)
(3) Cap tightly and vortex the sample for 10 seconds at maximum speed.
(4) Place the test tube in a tube stand in boiling water for 20 minutes.
(5) Cool the tube by placing it in an ice bath (until it becomes < 20ºC, 10 minutes).
(6) Suspend the sample well using a clean spatula. Place it back in the ice bath for 5 minutes to separate the solid matter and the liquid matter. The solid matter includes fat.
(7) Filter the contents through folded filter paper with a plastic funnel into a clean plastic tube; or centrifuge at 10,000 x g for 5 minutes and transfer the supernatant to a clean tube taking care not to disturb the pellet.
(8) The sample solution is now ready to assay.

Note: Be sure to perform the spike and recovery test written on page 3 for all samples.

2. Preparation of the Reagent

(1) Colorimetric solution:
Colorimetric reagent is in magenta-labeled vial. Remove the rubber plug very slowly so as not to lose any of the reagent powder. Add exactly 11 mL of distilled water to the vial. Swirl the vial gently so as not to produce foam until the contents are completely dissolved. Since the colorimetric reagent is extremely sensitive to the natural sunlight, handle the reagent in a place protected from the natural sunlight. Keep the solution between 0ºC and 10ºC. One vial of colorimetric reagent can be used for 10 assays under normal conditions.
It is recommended to use up the solution at one time, but if not possible, you may store it between 2ºC and 8ºC up to one week or keep it at -10ºC or below up to one month. Freezing and thawing are recommended to be done up to three times. When thawing the solution, use running water and thaw it as quickly as possible, and then keep it below 10°C.
(2) Enzyme solution:
Enzyme reagent is in green-labeled vial. Remove the rubber plug very slowly not to lose any the reagent powder. Add exactly 6 mL of the buffer (pink-labeled vial) to the vial. Swirl the vial gently so as not to produce foam until the contents are completely dissolved. Keep the solution between 0ºC and 10ºC. One vial of enzyme reagent can be used for 10 assays under normal conditions.
It is recommended to use up the solution at one time, but if not possible, you may keep it at -10ºC or below up to one month. Freezing and thawing are recommended to be done up to three times. When thawing the solution, use running water and thaw it as quickly as possible, 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 solution and the enzyme solution. Mix well and incubate at 37 ± 1ºC for 15 ± 2 minutes. Do not irradiate with strong light, especially sunlight during this series of operations. 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 0.85 ± 0.1 when using a spectrophotometer which has an optical path length of 2 cm or 0.5 ± 0.1 when using a spectrophotometer which has an optical path length of 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 when using a spectrophotometer with an optical path length of 2 cm or less than 0.05 when using a spectrophotometer with an optical path length of 1 cm under normal conditions. If not, check the operation procedure and perform the assay again.

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 solution	0.5	0.5	0.5	0.5
Enzyme solution	0.5	–	0.5	–
Buffer	–	0.5	–	0.5
	Es	Eb	Estd	Ec

Absorbance measurement conditions
Wavelength: 470 nm
Reference to set the absorbance to zero: distilled water
Final volume: 1.5 mL

4. Interpretation of Results

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

Histamine concentration (mg/kg)
= (Es – Eb) ÷ (Estd – Ec) × 4 × 25 × (100 ÷ Recovery rate(%))

Es: Absorbance of the sample; Eb: Absorbance of the sample blank; Estd: Absorbance of the standard solution; Ec: Absorbance of the reagent blank. Recovery rate is determined by the spike test as below. Values “4”, “25” and “100” in the formula mean that the histamine concentration of the standard solution is 4 mg/kg, the sample of raw tuna or canned tuna has been diluted 25 fold by extraction procedure, and the maximum recovery rate is 100 %, respectively.

Protocol for Spike and Recovery Tests

The recovery rate can be determined by conducting the following spike and recovery test. Note that the recovery rate may be very low when processed or fermented foods are used as samples. Therefore, the following test is required to confirm the recovery rate.

Note: Not all reagents and equipment required in this section are described in “Instruction for Use”.

1. Preparation of Histamine Solution (1,000 mg/kg) for Spike Test

Place histamine dihydrochloride in desiccators (without heat) for at least 2 hours to dry. Then weigh 167 mg of the dried histamine dihydrochloride, dissolve it in 0.1N HCl, and adjust to 100 mL in a 100 mL plastic graduated cylinder.

2. Preparation of Sample Solution

(1) Homogenize at least 10 g of the food stuff.
(2) Prepare two heat-resistant conical tubes with caps (tubes A and B). Weigh out precisely 1.0 g of the homogenized sample and transfer to tube A and B respectively.
(3) Add 0.1 mL of the histamine solution (1,000 mg/kg) to tube A. Add 0.1 mL of distilled water to tube B.
(4) Add 24.0 mL of sample treatment buffer to tube A and B. Cap both tubes tightly and vortex the sample for 10 seconds at maximum speed. Put the tubes in a tube stand. Boil it for 20 minutes. (This is a 25-fold dilution of the sample. The spiked histamine concentration after the dilution is approximately 4 mg/kg.)
(5) Cool the tubes by placing them in an ice bath (until it becomes < 20ºC, 10 minutes).
(6) Suspend the sample well using a clean spatula.
(7) Place it back in the ice bath for 5 minutes to separate the solid matter and the liquid matter.

(8) Filter the contents of (7) through folded filter paper into clean plastic tubes A and B, respectively; or centrifuge (10,000 x g, 5 minutes) and collect the supernatant to clean tubes A and B, respectively. The tubes are referred to as sample solution A and sample solution B, respectively.

Note: This recovery test can be evaluated at other spike levels by preparing the spike solution at a higher or lower concentration (e.g. 500 mg/kg) and adjusting the calculation of recovery rate accordingly.

Collect the homogeneous supernatant right away without disturbing the pellet, if the sample is centrifuged.

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." Perform measurements in triplicate and calculate the mean value.

4. Calculation of Recovery Rate

Determine the recovery rate of the sample by the following calculation:

Recovery rate (%) = (Conc. A – Conc. B) ÷ 100 mg/kg x 100%

Conc. A: Histamine concentration (mg/kg) in the sample solution A (with spiking 100 mg/kg histamine), Conc. B: Histamine concentration (mg/kg) in the sample solution B (without spiking histamine), 100 mg/kg: Histamine concentration spiked in tube A. For the determination of recovery rate, calculate the histamine concentration considering the recovery rate to be 100%.

Disposal Methods

The vessels of Colorimetric reagent and Enzyme reagent are consisted of glass, rubber and polypropylene cap. The vessels of the buffer and the histamine standard solution are polyethylene with a 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.
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 an allergic-like reaction.
5. Wear protection gloves when washing equipment 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". Do not 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. The expiration date is printed on the side of the box.

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.

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