Kit for the quantitative analysis of histamine

Histamine Test

For minimum 60 assays

User's manual

Histamine Test is a colorimetric enzyme assay for the quantitative analysis of histamine in canned, fresh or frozen fish.

- Easy extraction: Do not need to remove substances that interfere 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 enzyme 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 at 460 nm.

Product Specifications

1. No interference is seen with other amines, such as putrescine and cadaverine.
2. Sample range of quantitation: 20 ppm – 300 ppm
3. Testing time: 20 minutes

Storage of Kit

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

Composition of Kit

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

The kit contains reagents for minimum 60 assays using 400 µl enzyme per sample

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. As 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 or 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 well to separate solid phase and liquid phase.
(9) Freeze the samples if you do not assay them immediately. Only once freezing and thawing are recommended. While thawing the samples, keep them below 10°C. Be careful for the contamination of microorganisms during thawing the samples.
(10) Accurate time incubation is required. Otherwise you may not get correct result.
(11) Close the cap of the histamine standard solution immediately to avoid evaporation.

Instructions for Use

1. Materials Required But Not Provided

1. Homogenizer
2. Scale (which is able to weigh 1 g)
3. Heat-resistant plastic test tube with cap, which can cover tightly (50 ml tube, for extraction)
4. Pipette (-1 ml; to measure 0.4 ml and 1 ml) and tips

5. Distilled water
6. Paper filters and funnels (or Centrifuge)
7. Spectrophotometer (460 nm)
8. Plastic test tubes (for dilution of histamine standard solution and colorimetric assay reaction) and test tube racks
9. If available: Incubator (37°C)

Optional Items: (for raw material sample)

1. Heater (ex. gas stove) and pot
2. Sample treatment buffer:
   0.1 M EDTA·2Na (pH 8.0) solution
3. Ice

2. Extraction and Preparation of the Sample Solutions

<For Canned Fish>

1) Weigh about 10 grams of the sample 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 distilled water. Sample is diluted precisely 25 fold by above operations.
3) Suspend the fish tissue well.
4) Filter the contents through folded filter paper into a clean plastic tube. Or you can centrifuge (10,000 x g, 5 min) and collect the supernatant.
5) The sample solution is now ready to assay.

<For Raw material>

1) Weigh about 10 grams of the sample 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 precisely 25 fold by above operations.
3) Cover the cap tightly and suspend the fish tissue 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 fish tissue well 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 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.

3. Preparation of the Reagent

1. Colorimetric reagent:
   Colorimetric reagent is kept under vacuum in magenta-labeled vial. Add exactly 9 ml of distilled water. Stir the vial gently so as not to produce foam until the content are completely dissolved. Protect from light and keep below 4ºC before you use. One vial of colorimetric reagent can be used for 10 assays under normal condition.
2. Enzyme reagent:
   Enzyme reagent is kept under vacuum in green -labeled vial. Add exactly 5 ml of distilled water. Stir the vial gently so as not to produce foam until the content are completely dissolved. Keep below 4ºC before you use. One vial of enzyme reagent can be used for 10 assays under normal condition.

4. Assay Procedure

Refer to the table “Assay Procedure” below.
Incubate all the assay tubes simultaneously.

1. To assay N samples, prepare (2N+2) plastic test tubes.
2. To carry out sample assay, add 1.0 ml of distilled water and extracted sample solution. Then add 0.4 ml each of buffer, colorimetric reagent, and enzyme solution. Mix well and incubate at 25-37ºC 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 460 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.
3. To carry out sample blank assay, add 0.4 ml of distilled water instead of enzyme solution. Carry on the same operation as in 2. Measure the absorbance at 460 nm (Eb value).
4. To carry out histamine standard assay, add 1.0 ml of histamine standard solution instead of extracted sample solution. Carry on the same operation as in 2. Measure the absorbance at 460 nm (Estd value). The Estd value should be 0.45± 0.1 under normal conditions. If not, check the operation procedure and perform the assay again.
5. To carry out reagent blank assay, add 1.4 ml of distilled water instead of extracted sample solution and enzyme solution. Carry on the same operation as in 2. Measure the absorbance at 460 nm (Ec value). The Ec value should be <0.05 under normal conditions. If not, check the operation procedure and perform the assay again.

Absorbance measurement conditions
Wavelength: 460 nm
Cuvette: 1 cm path length
Reference to set the absorbance zero: water
Final volume: 3.2 ml

Table Assay Procedure

	Absorbance of the sample	Absorbance of sample blank	Absorbance of standard solution	Absorbance of reagent blank
Distilled water	1.0	1.0	1.0	1.0
Extracted sample solution	1.0	1.0	–	–
Histamine standard solution	–	–	1.0	–
Distilled water	–	–	–	1.0
Buffer	0.4	0.4	0.4	0.4
Colorimetric reagent	0.4	0.4	0.4	0.4
Enzyme solution	0.4	–	0.4	–
Distilled water	–	0.4	–	0.4
	Es	Eb	Estd	Ec

5. Interpretation of Results

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

The histamine concentration (mg/L = ppm)
$= (Es - Eb) \div (Estd\text{-}Ec) \times 4 \times 25 \times df$
$= (Es - Eb) \div (Estd\text{-}Ec) \times 100 \times df$

Es: Absorbance of the sample, Eb: Absorbance of sample blank, Estd: Absorbance of standard solution, Ec: Absorbance of reagent blank, df: dilution factor of the sample solution. Figure 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.

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 use by quality control personnel and others familiar with histamine analysis in fish.
3. 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.
4. 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.
5. Keep this kit away from children and infants.

Warranty

Hyserve 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, Hyserve 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 shall not be liable for any damages, including special or consequential damages, or expenses arising directly or indirectly from the use of this product.