



HISTAMARINE

ENZYME IMMUNOASSAY KIT Ref. IM2369

Instructions for the quantitative determination of histamine in fresh, frozen or canned fish by fish processors and quality control laboratories.



Samples of this test kit model were independently evaluated by the AOAC Research Institute and were found to perform to the producer's specifications as stated in the test kit's descriptive insert. The producer certifies this kit conforms in all respects to the specifications originally evaluated by the AOAC Research Institute as detailed in the *Performance Tested*SM Certification Number 980802.



INTENDED USE

The "Histamarine" enzyme immunoassay kit is intended for the quantitative determination of histamine concentrations in fresh, frozen or canned fish by fish processors or quality control laboratories.

It has been shown by the AOAC Research Institute-independent laboratory evaluation that the Histamarine test kit detects the presence of histamine in tuna, canned tuna and mahi-mahi.

The intended user should be familiar with enzyme immunoassays.

INTRODUCTION

Histamine, 5-imidazole-ethylamine, (MW = 111) is produced by the decarboxylation of free histidine, an amino acid present in muscle. Decarboxylation of histidine by enterobacteria may be rapid, if storage conditions are not satisfactory. Histamine is not destroyed by industrial sterilization, therefore, increased histamine levels are indicative of temperature abuse during handling and processing.

Histamine intake with food may lead to pseudo-allergic reactions. It is important therefore that concentrations are checked regularly during production as well as in the finished product.

PRINCIPLE OF THE ASSAY

Histamine contained in a fish sample is determined by use of high affinity specific monoclonal antibodies coated to the wells in a microtiter plate. The amount of histamine bound to antibodies is measured by use of enzyme conjugate which catalyzes a color change in the plate wells. The intensity of color is inversely proportional to the concentration of histamine in the sample. The concentration is calculated by use of a calibration curve obtained with calibrators supplied with the kit.

GENERAL REMARKS

The Histamarine kit may be used, in a single pass, to screen for histamine content in fish over a wider range (1–500 ppm for the Histamarine kit vs. 15–50 ppm for the 980802 AOAC method). Additional dilution may be required to obtain highest accuracy at levels at or above 200 ppm, since the Histamarine kit may overestimate at these high histamine levels.

The contents of each vial must be fully dissolved just before use.

Collection and handling of samples:

Histamine adsorbs to glass; therefore employ only plastic pipets and tubes.

Histamine is present in many biological materials; therefore wear gloves so as to avoid contamination of samples by, for example, sweat.

Enzyme immunoassay:

Do not mix reagents from different lots.

Let the components of the kit equilibrate to room temperature before use.

REAGENTS PROVIDED

All reagents in the unopened kit are stable, if stored at 2–8°C, until the expiration date stated on the kit. See description of individual kit components, listed below, for additional storage information.

Reagents of the kit may be labelled with IVD symbol, this is due to manufacturer internal purposes only. Do not take the IVD symbol into account. This kit is intended for the quantitative determination of histamine in fresh, frozen or canned fish by fish processors and quality control laboratories, not for use in diagnostic procedures.

Microtiter plate with lid: twelve strips of 8 detachable wells, coated with monoclonal anti-histamine antibody (ready-to-use).

Unused strips may be stored at 2–8°C in the self-lock bag provided.

Calibrators: five 1 mL vials (ready-to-use).

The five calibrator vials contain acylated histamine in buffer and cover a range from 0.1 to 50 µM (1 to 500 ppm). They contain 20 % DMSO (See Precautions).

Stability after opening: until expiration date of the kit if stored at 2–8°C. Carefully check that calibrators are completely thawed and homogeneous prior to use.

Acylation reagent: two vials (powder).

The contents of each vial must be dissolved just before use in the volume of DMSO stated on the vial label. (If several vials are needed, pool after dissolution, mix well).

Stability after dissolution: until expiration date of the kit if stored at –20°C. Contents must be completely thawed and homogeneous prior to use. Avoid repeated freezing and thawing.

DMSO (Dimethyl sulfoxide): two 3 mL vials

(ready-to-use).

Let this solvent equilibrate at room temperature until completely melted. (See Precautions).

Stability: until expiration date of the kit at 2–8°C.

Acylation buffer: four 5 mL vials (ready-to-use).

Stability: until expiration date of the kit at 2–8°C.

Histamine-alkaline phosphatase conjugate: 2 vials,
(contents lyophilized).

Reconstitute vials contents with the volume of distilled water indicated on the vial labels. Wait 20 min. before mixing. Pour reconstituted conjugates into Vial for conjugate and write on its label conjugate lot and diluted conjugate expiration.

Stability after reconstitution: one week at 2–8°C or until the expiration date of the kit if aliquoted and stored at –20°C.

Avoid repeated freezing and thawing.

Wash solution (20 X): one 50 mL vial.

This stock solution must be diluted to one liter with distilled water before use.

Stability after dilution: one month at 2–8°C, or until expiration date of the kit, stored at –20°C.

Substrate buffer: one 30 mL vial, (ready-to-use).

The substrate buffer is used to prepare the substrate working solution. It is a pH 9.8 diethanolamine HCl solution. It is irritant, observe proper precaution

Stability: until the expiration date of the kit at 2–8°C.

Substrate: two 15 mg tablets.

Para-nitrophenylphosphate (pNPP)

The substrate working solution is prepared just before use by dissolving the tablets in the substrate buffer: dissolve 1 tablet in 15 mL of buffer

Stability of the solution: 24 hours at 2-8 °C, or until the expiration date of the kit if aliquoted and stored at -20°C.

Contact with skin may affect product adversely. Handle tablets with forceps.

Stop solution: one 6 mL vial (ready-to-use).

This is a 1N NaOH solution; it is corrosive, observe proper precautions.

Stability: until the expiration date of the kit at 2-8°C.

Vial for conjugate: 1 vial

To be used for mixing of reconstituted conjugates.

MATERIALS AND REAGENTS REQUIRED BUT NOT PROVIDED

In addition to standard laboratory equipment, the following items are required:

precision balance, blender, microtiter plate reader (405-414 nm), variable volume precision micropipets (20 µL, 200 µL, 1000 µL), graduate cylinder 25 mL and 1L, plastic tubes, distilled water.

Centrifuge (10,000 x g), folded filter paper, plastic funnel and automatic microtiter plate washer are optional.

PRECAUTIONS**GHS HAZARD CLASSIFICATION****Stop solution**

DANGER

H314 Causes severe skin burns and eye damage.

P280 Wear protective gloves, protective clothing and eye/face protection.

P301+P330+P331 IF SWALLOWED: rinse mouth. Do NOT induce vomiting.

P303+P361+P353 IF ON SKIN (or hair): Rinse skin with water.

P304+P340 IF INHALED: Remove person to fresh air and keep at rest in a position comfortable for breathing.

P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P310 Immediately call a POISON CENTER or doctor/physician.

Sodium Hydroxide < 5%

**Substrate Buffer**

DANGER

H316 Causes mild skin irritation.

H318 Causes serious eye damage.

H373 May cause damage to organs through prolonged or repeated exposure.

P280 Wear protective gloves, protective clothing and eye/face protection.

P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and



easy to do. Continue rinsing.

P310 Immediately call a POISON CENTER or doctor/physician.

Diethanolamine 5 - 10%

Calibrators DANGER

H316 Causes mild skin irritation.

H360 May damage fertility or the unborn child.

P201 Obtain special instructions before use.

P280 Wear protective gloves, protective clothing and eye/face protection.

P308+P313 IF exposed or concerned: Get medical advice/attention.

P332+P313 If skin irritation occurs: Get medical advice/attention.

P405 Store locked up.

P501 Dispose of contents/container in accordance with local/national regulations

Dimethyl Sulfoxide 3-5%

Boric Acid 2-5%

**Alkaline phosphatase conjugate**

DANGER

H316 Causes mild skin irritation.

H360 May damage fertility or the unborn child.

P201 Obtain special instructions before use.

P280 Wear protective gloves, protective clothing and eye/face protection.

P308+P313 IF exposed or concerned: Get medical advice/attention.

P332+P313 If skin irritation occurs: Get medical advice/attention.

P405 Store locked up.

P501 Dispose of contents/container in accordance with local/national regulations

Boric Acid 0.1-0.5%

Zinc Chloride 0.1-0.5%

**DMSO WARNING**

H227 Combustible Liquid

H315 Causes skin irritation.

H319 Causes serious eye irritation.

H335 May cause respiratory irritation.

P210 Keep away from heat, hot surfaces, and sparks. No smoking.

P261 Avoid breathing vapours.

P271 Use only outdoors or in a well-ventilated area.

P280 Wear protective gloves, protective clothing and eye/face protection.

P302+P352 IF ON SKIN: Wash with plenty of soap and water.

P304+P340 IF INHALED: Remove person to fresh air and keep at rest in a position comfortable for breathing.

P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.



P312 Call a POISON CENTER or doctor/physician if you feel unwell.

P332+P313 If skin irritation occurs: Get medical advice/attention.

P337+P313 If eye irritation persists: Get medical advice/attention.

P362+P364 Take off contaminated clothing and wash it before use.

P370+P378 In case of fire: Use water spray for extinction.

P403+P233 Store in a well-ventilated place. Keep container tightly closed.

P403+P235 Store in a well-ventilated place. Keep cool.

P405 Store locked up.

P501 Dispose of contents/container in accordance with local/national regulations

Dimethyl Sulfoxide 100%

Acylation Buffer DANGER

H360 May damage fertility or the unborn child.

P201 Obtain special instructions before use.

P280 Wear protective gloves, protective clothing and eye/face protection.

P308+P313 IF exposed or concerned: Get medical advice/attention.

Boric Acid < 5%



Wash solution (20x) DANGER

H360 May damage fertility or the unborn child.

P201 Obtain special instructions before use.

P280 Wear protective gloves, protective clothing and eye/face protection.

P308+P313 IF exposed or concerned: Get medical advice/attention.

Boric Acid 0.1-0.3%

Sodium Borate Decahydrate 0.1-0.3%



ASSAY PROCEDURE

It is suggested that calibrators and samples be assayed in duplicate.

Calibrators and samples must be assayed at the same time.

Reagent preparation

Reconstitute the conjugate and dissolve the acylation reagent and prepare the wash solution and the substrate as indicated in section REAGENTS PROVIDED

Sampling and Extraction

Users must follow the regulatory requirements for fish sampling in their respective countries for the selection and number of samples. For example, two methods are provided below for preparation of the collected fish samples in the absence of stated national requirements:

1. Canned tuna fish (AOAC 937.07b). Place entire contents of can (meat and liquid) in blender and blend until homogeneous.

2. Fresh or thawed frozen whole fish (AOAC 937.07a). Large fish; clean and eviscerate 3 fishes, cut 3 cross-sectional pieces, 2.5 cm thick, from the back of pectoral fin,

halfway to vent, and posterior to vent. Debone slices and grind combined samples three times. For small fish ≤ 15 cm, clean, scale and eviscerate, place fish in blender and blend until homogeneous.

Obtain an amount of homogenized fish of between 1 and 10 grams. Weigh it precisely in a pre-weighed plastic tube. Extract histamine with 8 milliliters of distilled water per gram of tissue by grinding to homogeneity in a blender. Centrifuge at 10,000 x g for 5 min. or filter through folded paper placed in a plastic funnel or decant, carefully collecting supernatant. Dispense supernatant fluid in a plastic tube.

Acylation of samples

In a clean plastic tube, dilute 20 μ L of supernatant in 180 μ L of acylation buffer.

Add 50 μ L of acylation reagent to all tubes containing dilutions of the extracts (200 μ L). Homogenize reaction mixture well. Acylation is instantaneous.

Acylated histamine is quite stable. After acylation, samples may be kept at 2-8°C for 48 h. Let them equilibrate at room temperature, mix well before assay.

Immunoassay

To each antibody-coated well add 50 μ L of acylated calibrator or sample followed by 200 μ L of enzyme conjugate.

Incubate for 30 min at 18-25°C.

Washing

Wash the microtiter plate wells using one of the washing procedures described below. It is essential to remove all unbound components in any enzyme immunoassay to avoid high background.

The wash procedure must not be interrupted. Do not let wells dry out prior to the addition of substrate.

Automated procedure

Wash the microtiter plate wells 3 times, using a microtiter plate washer that meets the following criteria:

- The fluid in the wells must be completely aspirated.
- The wells must be filled to the rim with the wash solution.
- The wash solution must be injected rapidly; (approximately one second to fill each well).

Alternatively a manual wash procedure may be used.

Manual procedure

- Turn microtiter plate upside-down and shake vigorously over the sink.
- Use a squeeze bottle to fill the wells with wash solution. The solution may run over the rim of the wells.
- Repeat a., b. and a. once more for a total of two washes.
- Firmly tap the upside-down microtiter plate on clean absorbant paper.

Enzymatic step

Add 200 μ L of substrate to all wells, the dispensing should not take more than 3 min.

Cover plate with lid and incubate for 30 min at 18 - 25°C.

After incubation, stop the reaction by adding 50 μL of stop solution to all wells. Pipeting time for the stop solution must be equivalent to pipeting time for substrate.

Reading

Using the spectrophotometer, set to between 405 to 414 nm, read the wells.

Reading may be delayed for up to a maximum of 2 hours, if the plate is kept covered with its lid in order to avoid evaporation.

Summary of the assay procedure

This is only a summary. The above procedure should be read thoroughly to ensure that proper techniques are used.

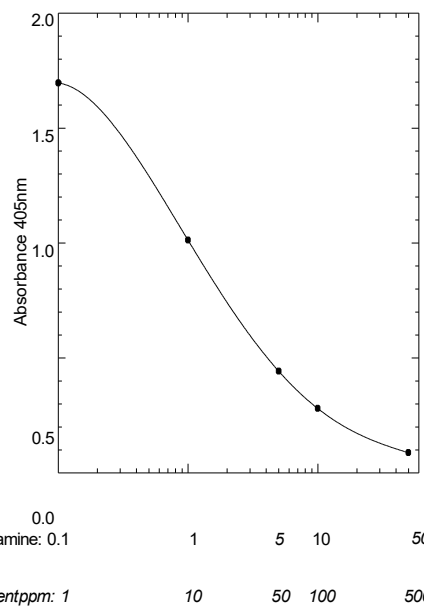
Extraction	Samples	Immuno assay	Results
<p>Weigh precisely the fish sample (between 1 and 10 grams).</p> <p>Crush to homogeneity in 8 mL of water per gram of fish in a blender.</p> <p>Centrifuge (10,000 x g 5min) or filter (paper filter) or decant (carefully collect supernatant).</p>	<p>In plastic tubes containing 180 μL of acylation buffer dispense 20 μL of extraction supernatant.</p> <p>Add 50 μL of acylation reagent, mix.</p> <p>At this step, samples may be stored 48 h at 2-8°C.</p>	<p>To plate wells add 50 μL of sample or of ready to use calibrator</p> <p>Add 200 μL of conjugate.</p> <p>Incubate 30 min at 18-25°C.</p> <p>Wash wells carefully.</p>	<p>To plate wells add 200 μL of substrate and incubate 30 min at 18-25°C.</p> <p>Add 50 μL of stop solution.</p> <p>Read plate at 405-414 nm.</p>

RESULTS

Results are obtained by interpolation from standard curve.

Using the absorbance of the 5 calibrators provided with the kit, draw a standard curve on semi-log paper, or use appropriate software plotting on the horizontal axis (log scale) the histamine concentration of the 5 calibrators and on the vertical axis (linear scale) the corresponding absorbance value obtained for each calibrator.

When the points have been plotted for each histamine calibrator, connect points resulting in a standard curve. An example of how the curve should appear is presented here.



Example of standard curve, do not use for calculation. Use the concentration of calibrators indicated on each vial label. The concentrations are lot specific, check carefully.

Using the standard curve, locate absorbance on vertical axis and read off corresponding histamine concentrations on horizontal axis.

A concentration of 1 μM read on the horizontal axis corresponds to a histamine concentration of 10 ppm (10 mg/kg) in the original tissue sample. This correspondency takes into account dilutions performed in sample preparation.

PERFORMANCE

The sensitivity, defined as the lowest concentration of acylated histamine significantly different from zero with a probability of 95 %, is 0.03 μM (0.3 ppm).

At the 1 μM concentration, 1-methyl histamine, 3-methyl histamine, putrescine and histidine do not show cross-reactivity higher than 0.05 % in the assay.

Recovery experiments performed on fish samples gave an average recovery of 103.5 %.

Intra-assay

Samples were assayed 25 times in the same series. The coefficients of variation were found below or equal to 8.91%.

Inter-assay

Samples were assayed in duplicate in 10 different series. The coefficients of variation were found below or equal to 17.16 %.

EXPECTED VALUES

Depending on local legislation, histamine levels must be below 50-200 ppm (50-200 mg/kg).























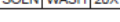



TECHNICAL ASSISTANCE

In case of packaging deterioration or if data obtained show some performance alteration, please contact your local distributor or use the following e-mail address: imunochem@beckman.com.



APPENDIX

Symbols Key

Symbols Key	
	Danger
	Product Reference
	In Vitro Diagnostic
	Contents
	Manufactured By
	Contains sufficient for <n> tests
	Safety Data Sheets
	Consult Instructions for Use
	Temperature Range(s)
	Caution
	Expiration Date
	Lot Number
	Date of Manufacture
	Biohazard
	Calibrator
	Plate
	Instructions for Use
	Acylation Reagent
	Acylation Buffer
	Substrate Buffer
	Dimethyl Sulfoxide
	Conjugate
	Wash Solution (20X)
	Stopping Solution
	Substrate
	Vial for conjugates