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Please use only the valid version of the Instructions for Use provided with the kit

Instructions for use

HistaSure ™ ELISA Fast Track

PERFORMANCE TESTED
AOAC
RESEARCH INSTITUTE
LICENSE NUMBER 021402

REF **FC E-3600**

2°C – 8°C

Σ 48

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1. Intended use and principle of the test

The **HistaSure ™ ELISA** Fast Track is intended for the rapid semi-quantitative or quantitative determination of histamine in fresh/frozen tuna, canned tuna, frozen mahi-mahi, canned sardines and fish meal.
The ELISA may also be used for the measurement of histamine in other scombroid fish types; however, such applications are outside the scope of the AOAC validation.

Related Products:

HistaSure ™ XL ELISA Fast Track FC E-3900 (480 determinations)

The assay kit provides materials for the determination of derivatized histamine in food extracts. The derivatization is part of the preparation of the samples. By use of the acylation reagent, histamine is quantitatively derivatized into N-acylhistamine. The competitive Histamine ELISA kit uses the microtiter plate format. Histamine is bound to the solid phase of the microtiter plate. Acylated histamine and solid phase bound histamine compete for a fixed number of antiserum binding sites. When the system is in equilibrium, free antigen and free antigen-antiserum-peroxidase complexes are removed by washing. The substrate TMB/peroxidase reaction is monitored at 450 nm. The amount of antibody bound to the solid phase histamine is inversely proportional to the histamine concentration of the sample.

2. Introduction

Histamine testing in fresh fish is a possible control strategy that can be used by seafood processors in their HACCP program to address the hazard of scombrotoxin formation. Histamine is a product of decomposition of histidine caused by the growth of certain bacteria in seafood. The amount of the amine that forms is a function of bacterial species, the temperature and time of exposure, and may exceed 1,000 ppm (mg/kg). Fish containing high levels of histamine has been associated with many examples of poisoning commonly referred to as "scombroid poisoning," a major health problem for consumers. Scombrotoxic fish usually contains levels of histamine in excess of 200 ppm but such fish may be randomly dispersed within a lot. For large fish, histamine is found at variable levels even within individual fish. Quality control measures designed to minimize the occurrence of scombrotoxic fish require the determination of histamine levels in the range of approximately 10 to 200 ppm. Good quality fish contain less than 10 ppm histamine, a level of 30 ppm indicates significant deterioration, and 50 ppm is considered to be evidence of definite decomposition. The defect action level (DAL), the level at which regulatory actions are taken for histamine is 50 ppm (P. L. Rogers, W. F. Staruszkiewicz, Journal of Aquatic Food Product Technology, Vol. 9 (2) 2000 p. 5 – 17).

3. Procedural cautions, guidelines and warnings

(1) This kit is for professional use only. Users should have a thorough understanding of this protocol for the successful use of this kit. Only the test instruction provided with the kit is valid and has to be used to run the assay. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
(2) In order to reduce exposure to potentially harmful substances, wear lab coats, disposable latex gloves and protective glasses where necessary.
(3) All kit reagents and specimens should be brought to room temperature (20 – 25 °C) and mixed gently but thoroughly before use.
(4) When the use of water is specified for dilution or reconstitution, use deionized, distilled, or ultra-pure water.
(5) The microplate contains snap-off strips. Unused wells must be stored at 2 – 8 °C in the sealed foil pouch and used in the frame provided. Wells are for single use only.
(6) Once the test has been started, all steps should be completed without interruption. Make sure that required reagents, materials and devices are prepared ready at the appropriate time.
(7) Incubation times do influence the results. All wells should be handled in the same order and time sequences.
(8) To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard and control.
(9) Do not mix various lot numbers of kit components within a test and do not use reagents beyond expiry date as shown on the kit labels.
(10) Some reagents contain sodium azide (NaN_3) as preservatives. In case of contact with eyes or skin, flush immediately with water. NaN_3 may react with lead and copper plumbing to form explosive metal azides. When disposing reagents, flush with a large volume of water to avoid azide build-up.
(11) For information on hazardous substances included in the kit please refer to Safety Data Sheets (SDS). The Safety Data Sheet for this product is available directly on the website of the manufacturer or upon request.
(12) Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.
(13) In case of any severe damage to the test kit or components, the manufacturer has to be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components must not be used for a test run. They must be stored properly until the manufacturer decides what to do with them. If it is decided that they are no longer suitable for measurements, they must be disposed of in accordance with national regulations.

4. Storage and stability

Store the reagents at 2 – 8 °C until expiration date. Do not use components beyond the expiration date indicated on the kit labels. Do not mix various lots of any kit component within an individual assay.

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5. Materials

5.1 Contents of the kit

BA D-0035	MB 48	**Master Block** – ready to use
Content:	1 x 48 wells plate in a resealable pouch	
BA E-0030	WASH-CONC 50x	**Wash Buffer Concentrate** – concentrated 50x
Content:	Buffer with a non-ionic detergent and physiological pH	
Volume:	1 x 20 ml/vial, purple cap	
BA E-0055	SUBSTRATE	**Substrate** – ready to use
Content:	Chromogenic substrate containing 3,3',5,5'-tetramethylbenzidine, substrate buffer and hydrogen peroxide	
Volume:	1 x 12 ml/vial, black cap	
BA E-0080	STOP-SOLN	**Stop Solution** – ready to use
Content:	0.25 M sulfuric acid	
Volume:	1 x 12 ml/vial, grey cap	
FC E-3611	ACYL-BUFF	**Acylation Buffer** – ready to use
Content:	TRIS buffer with non-mercury preservative	
Volume:	2 x 50 ml/vial, white cap	
FC E-3612	ACYL-REAG	**Acylation Reagent** – ready to use
Content:	Acylation Reagent in DMSO, yellow coloured	
Volume:	1 x 3 ml/vial, white cap	
FC E-3631	HIS	**Histamine Microtiter Strips** – ready to use
Content:	1 x 48 wells (6x8) antigen precoated microwell plate in a resealable pouch with desiccant	
FC E-3640	HIS AB CONJ	**Histamine Antiserum Conjugate** – ready to use
Content:	Goat-anti Histamine IgG conjugated with peroxidase	
Volume:	1 x 6 ml/vial, red cap	
Hazard pictograms:	GHS07	
Signal word:	Warning	
Hazardous ingredients:	2-methyl-2H-isothiazol-3-one	
Hazard statements:	H317 May cause an allergic skin reaction.	
Precautionary statements:	P280 Wear protective gloves. P302+P352 IF ON SKIN: Wash with plenty of water. P333+P313 If skin irritation or rash occurs: Get medical advice/attention. P501 Dispose of contents/container to an authorised waste collection point.	

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5.2 Calibration and Controls

Controls – ready to use

Cat. no.	Component	Colour/Cap	Concentration	Volume/ Vial
FC E-3601	**C⇨0 PPM**	white	0 ppm	4 ml
FC E-3602	**C⇨3 PPM**	yellow	3 ppm	4 ml
FC E-3603	**C⇨10 PPM**	orange	10 ppm	4 ml
FC E-3604	**C⇨20 PPM**	green	20 ppm	4 ml
FC E-3605	**C⇨30 PPM**	purple	30 ppm	4 ml
FC E-3606	**C⇨50 PPM**	blue	50 ppm	4 ml
FC E-3607	**C⇨100 PPM**	grey	100 ppm	4 ml
FC E-3608	**C⇨300 PPM**	black	300 ppm	4 ml

Content: Ultrapure water with non-mercury stabilizer, spiked with a defined quantity of Histamine

5.3 Additional materials and equipment required but not provided in the kit

- Precision pipette (50 µl)
- Pipette tips (50 µl)
- Manual repetitive pipette (e.g. the Brand HandyStep® S)
- Precision Dispenser Tips (5 ml, 25 ml; e.g. the PLASTIBRAND® PD-Tips)
- Grinder (mill) or house hold blender
- Graduated plastic cylinder (250 ml)
- Water (deionized, distilled, or ultra-pure)
- Scale (capable of weighing 5 – 50 grams, precision 0.1 gram)
- Funnel and filter paper (or alternatively a centrifuge)
- Timer
- Waterproof marker
- Absorbent material (paper towel)
- Microplate Vibration Shaker (shaking amplitude 2 mm; approx. 600 rpm, (e.g. PSU-2T Minishaker*)
- ELISA reader capable of reading absorbance at 450 nm (required for semi-quantitative and quantitative determination)
- Washing device (plate washer or manually)

**Available upon request!*

6. Test procedure

6.1 Preparation of reagents

Wash Buffer

Dilute the 20 ml Wash Buffer Concentrate **WASH-CONC** **50X** with water to a final volume of 1000 ml.
Storage: up to 6 months at 2 – 8 °C

Acylation Reagent

The Acylation Reagent has a freezing point of 18.5 °C. To ensure that the Acylation Reagent is liquid when being used, it must be ensured that the Acylation Reagent has reached room temperature and forms a homogeneous, crystal-free solution before being used.

Histamine Microtiter Strips

In rare cases residues of the blocking and stabilizing reagent can be seen in the wells as small, white dots or lines. These residues do not influence the quality of the product.

6.2 Sample preparation

The following protocols for the sample preparations are based on the AOAC Official Method 937.07
Sampling should be performed according to national regulation.

A. FRESH FISH • FROZEN FISH

- Keep (fresh) fish frozen prior to analysis.
- Thaw samples under refrigeration or in cold water. Do not thaw the samples in a heated water bath. Discard draining.
- Once thawed, store the samples refrigerated (2 – 8 °C) prior to testing.

whole fish:

Clean, scale and eviscerate fish. In case of small fish 6 in. (≤ 15 cm), use 5 – 10 fish. In case of large fish, from each of ≥ 3 fish cut 3 cross-sectional slices 1 in. (2.5 cm) thick, 1 slice from just back of pectoral fins, 1 slice halfway between first slice and vent, and 1 slice just back of vent. Remove bone. Blend combined samples until homogenous.

fish filet:

Use entire piece. Blend until homogenous.

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B. CANNED FISH and other CANNED MARINE PRODUCTS
Place entire content of the can (meat and liquid) in a blender and blend until homogenous.

C. CANNED MARINE PRODUCTS PACKED in OIL, SAUCE, BRINE or BROTH
Drain for 2 minutes on number 8 sieve or dab away the fluid with a paper towel. Place the meat in a blender and blend until homogenous.

D. FISHMEAL
Mix sample until homogenous.

6.3 Extraction

- **Weigh 10 g** of prepared fish sample / fish meal, **add 240 ml water** (deionized, distilled, or ultra-pure) and **homogenize***) for 1 – 2 minutes in a grinder or blender.
  ):** Instead of homogenization ***fish meal samples are stirred for 10 minutes at room temperature.
- **Filter** the homogenate through folded filter paper (alternatively an aliquot of the homogenate can be centrifuged for 5 minutes at maximum speed). *If a **lipid layer** forms remove it by suction!*
- Use 50 µl of the **sample extract** for the acylation.

6.4 Histamine ELISA

For the subsequent steps (Acylation and ELISA) allow all reagents and samples to reach room temperature.

A. Semi-quantitative Determination
For the semi-quantitative determination select the desired cut-off you need from the controls provided with the kit. The kit controls have the following concentrations:
control 3, 10, 20, 30, 50, 100 or 300 ppm.

B. Quantitative Determination
For the quantitative determination use the following controls provided with the kit**:**
control 0, 3, 10, 30, 100 and 300 ppm.

These 6 controls are used to establish the standard curve (please refer to section 7.)

6.4.1 Acylation

1.	Pipette **50 µl** of **control(s)** and **sample extracts** into the respective wells of the **Master Block** **MB 48**.
2.	Add **1.5 ml** of **ACYL-BUFF** in **1 (!)** pipetting step to all wells. *The use of a repetitive pipette together with a new Precision Dispenser Tip (25 ml, please refer to chapter 5.), is recommended.*
3.	Add **50 µl** of **ACYL-REAG** to all wells. *(Colour change from yellow to pink!)* ⚠ ***Continue without any delay with step 4.!*** *The use of a repetitive pipette together with a new Precision Dispenser Tip (2.5 ml, please refer to chapter 5.), is recommended.*
4.	Incubate **5 min** at **RT** (20 – 25 °C) on a **shaker** (approx. 600 rpm). ⚠ ***Make sure that mixing is complete (slight pink colour).***
5.	Take **50 µl** for the ELISA.

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6.4.2 Histamine ELISA

1.	Pipette **50 µl** of the **acylated control(s)** and **samples** into the wells of the **Histamine Microtiter Strips** **W** **HIS**.
2.	Pipette **100 µl** of the **HIS** **AB** **CONJ** into all wells. *The use of a repetitive pipette together with a new Precision Dispenser Tip (5 ml, please refer to chapter 5.), is recommended.*
3.	Incubate **10 min** at **RT** (20 – 25 °C) on a **shaker** (approx. 600 rpm).
4.	Discard or aspirate the contents of the wells. Wash the plate **3 x** by adding **300 µl** of **Wash Buffer, discarding** the content and **blotting dry each time** by tapping the inverted plate on absorbent material.
5.	Pipette **100 µl** of the **SUBSTRATE** into all wells. *The use of a repetitive pipette together with a new Precision Dispenser Tip (5 ml, please refer to chapter 5.), is recommended.*
6. ⚠	Incubate for **10 min** at **RT** (20 – 25 °C) on a **shaker** (approx. 600 rpm). ***Avoid exposure to direct sunlight!***
7.	Add **100 µl** of the **STOP-SOLN** to each well and shake the microtiter plate to ensure a homogeneous distribution of the solution. *The use of a repetitive pipette together with a new Precision Dispenser Tip (5 ml, please refer to chapter 5.), is recommended.*
8.	**Read** the absorbencies of the solutions in the wells within 10 minutes using a **microplate reader** set to **450 nm**.

7. Calculation of results

A. SEMI-QUANTITATIVE RESULTS:
If the absorbance of the sample is **higher** than that of the selected **Cut-Off Control**, the sample has **passed**.

If the absorbance of the sample is **lower** than that of the selected **Cut-Off Control**, the sample has **failed**.

B. QUANTITATIVE RESULTS:
The absorbance readings of the 6 controls (0, 3, 10, 30, 100 and 300 ppm) are used to establish a standard curve. Plot the absorbance readings of the controls (y-axis, linear) against the corresponding control concentrations (x-axis, log) using a concentration of 0.001 ppm for the 0-control (*this alignment is mandatory because of the logarithmic presentation of the data*). For the curve fitting a non-linear regression has to be applied.

The concentrations of the samples can be read **directly** from this standard curve.

⚠ *If a sample is off-curve it has to be diluted with water 1:10 and re-assayed. The result obtained has to be multiplied by the dilution factor of 10.*

8. Warranty

This test kit was produced according to the latest developments in technology and subjected to stringent internal and external quality control checks. Any alteration of the test kit or the test procedure as well as the usage of reagents from different charges may have a negative influence on the test results and are therefore not covered by warranty. The manufacturer is not liable for damages occurring during transit.

9. Application lists for different kind of fish samples

All fish samples tested so far are suitable for the **HistaSure ™ ELISA Fast Track**. The lists below depict some major applications in different matrices.

	Fish species	Presentation
Species validated through AOAC Certification	Tuna	canned chunk light
		fresh/frozen yellow fin
	Mahi Mahi	frozen
	Sardines	canned in oil
	Fishmeal	

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	Fish species	Presentation
Species validated through in-house testing	Mackerel	smoked
	Anchovy	fresh
		brined
		in sauce
	Shad	dry salted
		fermented
	Herring	smoked
	Salmon	smoked
	Bonito	lakerda
	Swordfish	fresh
	Marlin	fresh

Fish products validated through in-house testing	Maldive fish
	Fish sauce (sardines/anchovy)
	Oyster sauce (oyster/sardines)

10. Assay characteristics

AOAC performance tested method for fresh/frozen yellowfin tuna, canned tuna-chunk light in water, frozen mahi mahi, canned sardines in oil and fish meal.

	Substance	Cross Reactivity (%) Histamine
Analytical Specificity (Cross Reactivity)	Histamine	100
	L-Tryptophan	nd
	Tryptamine	nd
	3-Methylhistamine	0.44
	L-Histidine	nd
	L-Tyrosine	nd
	L-Phenylalanine	nd
	Tyramine	0.69
	Cadaverine	0.40
	Spermine	nd
	Putrescine	nd
	Trimethylamine	nd
	nd = not detectable	

Accuracy and Precision					
Recovery				Intra CV	
Sample	**Fortification range (ppm)**	**Mean recovery (%)**	**Recovery range (%)**	**Mean CV (%) (n = 7)**	**Range CV (%)**
Fresh/Frozen Tuna	5.25 – 218.6	91.8	85.2 – 99.6	7.58	4.13 – 10.8
Canned Tuna	5.23 – 298.3	101.8	91.0 – 119	7.67	2.50 – 13.9
Frozen Mahi Mahi	6.38 – 199.0	87.3	79.1 – 103	6.19	2.97 – 9.56
Canned Sardines	5.27 – 249.9	87.6	76.8 – 99.7	5.65	1.80 – 9.22
Fish meal	9.4 – 244.2	86.0	79.0 – 93.9	4.89	2.09 – 7.92

	Mean (ppm)	n	Inter CV %
Lot-to-Lot	25.8	3 lots	4.16

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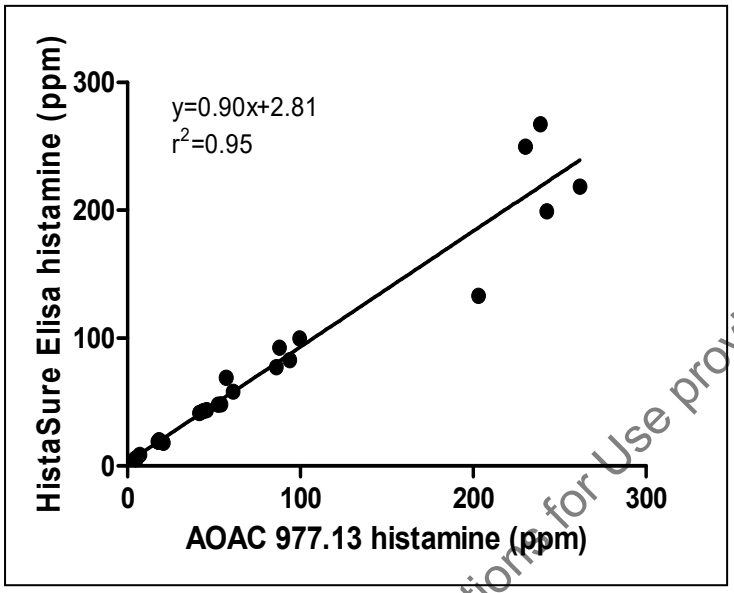
Detection limits	
LOD (Limit of Detection)	**LOQ (Limit of Quantification)**
0.44 ppm	1.31 ppm

Method Comparison

LDN HistaSure ELISA vs. AOAC 977.13 fluorometric method: Fresh/Frozen Tuna, Canned Tuna, Frozen Mahi Mahi and Canned Sardines



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Flow Chart HistaSure™ ELISA Fast Track FC E-3600 / FC E-3900
Quantitative / Semi-Quantitative

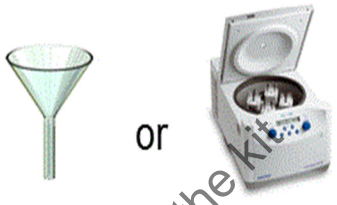
- For a quantitative result use the following controls provided in the kit: control 0, 3, 10, 30, 100 and 300 ppm
- For a semi-quantitative result select the desired cut-off you need from the controls provided with the kit

1. Extraction

- Weigh 10 grams of fish or fishmeal
- Add 240 ml of water
- Homogenize 1 -2 min. (fishmeal stir 10 min)

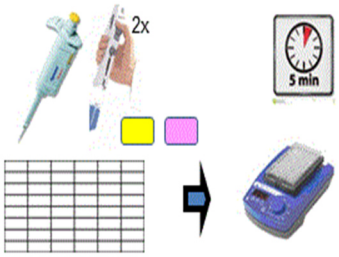
2. Filter or centrifuge

- Filter the homogenate or centrifuge an aliquot of the homogenate (5 min max speed)
- If a lipid layer forms, remove by suction!



3. Acylation

- Pipet 50 µl control(s) / sample extract into the masterblock
- Add 1.5 ml Acylation Buffer
- Add 50 µl Acylation Reagent
- Incubate for 5 min on a shaker



4. ELISA: step 1

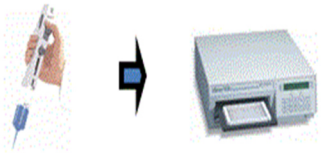
- Pipet 50 µl acylated control(s)/sample into the ELISA wells
- Add 100 µl Antiserum Conjugate
- Incubate for 10 min on a shaker

5. ELISA: step 2 and 3

- Discard the content of the wells and wash each well 3x
- Add 100 µl Substrate
- Incubate for 10 min on a shaker

6. ELISA: step 4

- Add 100 µl of Stop Solution
- Shake shortly
- Read the plate at 450 nm



Calculation of Results: Quantitative

- Plot the absorbance readings of the calibrators (y-axis, linear) against the corresponding calibrator concentrations (x-axis, log).

Control Histamine (ppm)	0.001	3.0	10	30	100	300

- Use non-linear regression for curve fitting
- Read the concentrations of the samples

Calculation of Results: Semi-quantitative

- If the absorbance of the samples > then that of selected cut-off, the sample has passed
- If the absorbance of the samples < then that of selected cut-off, the sample has failed

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⚠ ***The liability of the manufacturer shall be limited to the replacement of defective products. The manufacturer takes no liability for any damages or expenses arising directly or indirectly from the use of this product.***

Symbols:

+2 +8 °C	Storage temperature		Manufacturer	Σ	Contains sufficient for <n> tests
	Use-by date	LOT	Batch code		Distributor
	Consult instructions for use	CONT	Content		Date of manufacture
	Caution	REF	Catalogue number		Do not re-use