

Instructions for use
Histamine Food ELISA

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

REF**FC E-3100**

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Histamine Food ELISA

1. Principle of the test

Fish meal that has been produced from materials which has been allowed to degrade prior to being processed can contain high levels of histamine and can be toxic. Elevated histamine levels (1,000 ppm) can cause gizzard erosion and black vomit in poultry. 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 scombrototoxin 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. Scombrototoxic 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 scombrototoxic 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).

The assay kit provides materials for the quantitative 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 antibody binding sites. When the system is in equilibrium, free antigen and free antigen-antibody complexes are removed by washing. The antibody bound to the solid phase histamine is detected by anti-goat/peroxidase. 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. Procedural Cautions, Guidelines and Warnings

2.1 Procedural cautions, guidelines and warnings

- (1) This kit is intended 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) The principles of Good Laboratory Practice (GLP) have to be followed.
- (3) In order to reduce exposure to potentially harmful substances, wear lab coats, disposable latex gloves and protective glasses where necessary.
- (4) All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
- (5) For dilution or reconstitution purposes, use deionized, distilled, or ultra-pure water.
- (6) The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch with desiccant and used in the frame provided.
- (7) Duplicate determination of sample is highly recommended to be able to identify potential pipetting errors.
- (8) Once the test has been started, all steps should be completed without interruption. Make sure that the required reagents, materials and devices are prepared ready at the appropriate time.
- (9) Incubation times do influence the results. All wells should be handled in the same order and time intervals.
- (10) To avoid cross-contamination of reagents, use new disposable pipette tips for dispensing each reagent, sample, standard and control.
- (11) A standard curve must be established for each run.
- (12) The controls should be included in each run and fall within established confidence limits. The confidence limits are listed in the QC-Report provided with the kit.
- (13) Do not mix kit components with different lot numbers within a test and do not use reagents beyond expiry date as shown on the kit labels.
- (14) Avoid contact with Stop Solution containing 0.25 M H₂SO₄. It may cause skin irritation and burns. In case of contact with eyes or skin, rinse off immediately with water.
- (15) TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them.
- (16) For information on hazardous substances included in the kit please refer to Safety Data Sheet (SDS). The Safety Data Sheet for this product is made available directly on the website of the manufacturer or upon request.
- (17) Kit reagents must be regarded as hazardous waste and disposed according to national regulations.

2.2 Limitations

Any inappropriate handling of samples or modification of this test might influence the results.

2.3 High-Dose-Hook effect

No hook effect was observed in this test.

3. Storage and stability

Store the unopened reagents at 2 - 8 °C until expiration date. Do not use components beyond the expiry date indicated on the kit labels. Once opened the reagents are stable for 1 month when stored at 2 - 8 °C. Once the resealable pouch has been opened, care should be taken to close it tightly with desiccant again.

4. Materials

4.1 Contents of the kit

BA D-0024 REAC-PLATE **Reaction Plate** - Ready to use

Content: 1 x 96 well plate, empty 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, light purple cap

BA E-0055 SUBSTRATE **Substrate** - Ready to use

Content: Chromogenic substrate containing tetramethylbenzidine, substrate buffer and hydrogen peroxide

Volume: 1 x 12 ml/black 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, light grey cap

Hazards

identification:



H290 May be corrosive to metals.

BA E-1031 HIS **Histamine Microtiter Strips** - Ready to use

Content: 1 x 96 well (12x8) antigen precoated microwell plate in a resealable pouch with desiccant.

Standards and Controls Ready to use

Cat. no.	Component	Colour/Cap	Concentration ng/ml	Concentration nmol/l	Volume/ Vial
BA E-1001	STANDARD A	white	0	0	4 ml
BA E-1002	STANDARD B	light yellow	0.5	4.5	4 ml
BA E-1003	STANDARD C	orange	1.5	13.5	4 ml
BA E-1004	STANDARD D	dark blue	5	45	4 ml
BA E-1005	STANDARD E	light grey	15	135	4 ml
BA E-1006	STANDARD F	black	50	450	4 ml
BA E-1051	CONTROL 1	light green	Refer to QC-Report for expected value and acceptable range!		4 ml
BA E-1052	CONTROL 2	dark red			4 ml

Conversion: Histamine (ng/ml) x 9 = Histamine (nmol/l)

Histamine (ng/ml) = Histamine (µg/l) = Histamine (µg/kg) = Histamine (ppb)

Content: Acidic buffer spiked with defined quantity of Histamine

- BA E-1210** HIS-AS **Histamine Antiserum** - Ready to use
 Content: Goat anti-histamine antibody, blue coloured
 Volume: 1 x 12 ml/vial, blue cap
- BA E-1711** ACYL-BUFF **Acylation Buffer** - Ready to use
 Content: TRIS-buffer
 Volume: 1 x 22 ml/vial, brown cap
- BA E-1712** ACYL-REAG **Acylation Reagent** - Ready to use
 Content: Acylation reagent containing DMSO
 Volume: 1 x 3 ml/vial, green cap
- BA E-1040** CONJUGATE **Enzyme Conjugate** - Ready to use
 Content: Donkey anti-goat immunoglobulins conjugated with peroxidase
 Volume: 1 x 12 ml/vial, red cap

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

- Calibrated precision pipettes to dispense volumes between 25 – 200 µl
- ELISA plate reader capable of reading absorbance at 450 nm and if possible 620 - 650 nm
- Centrifuge capable of at least 3.000 x g
- Absorbent material (paper towel)
- Water (deionized, distilled, or ultra-pure)
- Vortex mixer
- for milk: precipitation reagent and 0.1 N hydrochloric acid (HCl)

Please note:

- The assay can be performed with or without the use of a shaker. If a shaker is used it should have the following characteristics: shaking amplitude 3 mm; capable of approx. 600 rpm.
- The washing steps can be performed manually or by the use of a microplate washing device.

5. Sample preparation of histamine from different sources

5.1 Application list for different kind of fish samples

All fish samples tested so far are suitable for the Histamine Food ELISA. The list below depicts some major applications in different matrices:

Fish Species	Presentation
Anchovy	Fresh with mediterranean sauce in brine (20 %, 25 %, 30 %)
Atlantic bonito	dry and salted fresh pickled
Blue fin tuna	fresh
Fer. Herring	Lekmogen
Fer. Herring	Eric den Rode
Fer. Herring	Lykeburg
Fer. Herring	Massens
Horse Mackerel	fresh
Mackerel	smoked pickled
Rainbow trout	fresh
Salmon	fresh
So-juy mullet	fresh
Tuna	canned

Different species	Fish meal
Different species	Fish paste

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.

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 both

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.

Mix 10 g of homogenized fish sample (A. – C.) and 90 ml of water (ultrapure) for 1- 2 minutes by use of a blender. Pipette 1 ml of the suspension into an Eppendorf-tube or similar centrifugation device and centrifuge for 5 minutes at maximum speed. Remove lipid layer by suction!

Take 20 μ l of the supernatant and dilute it with 10 ml of distilled water (*for this dilution step, do not use any glass ware!*).

Use 100 μ l for acylation!

D. FISH MEAL

Mix sample until homogenous.

Suspend 1 g of fish meal in 200 ml of distilled water and stir for 15 minutes. Pipette 1 ml of the suspension into an Eppendorf-tube or similar centrifugation device and centrifuge for 5 minutes at maximum speed. Take 20 μ l of the supernatant and dilute it with 20 ml of distilled water (*for this dilution step, do not use any glass ware!*).

Use 100 μ l for the acylation!

5.2 Sausage (processed, smoked or fermented meats)

Homogenize 10 g of sausage in 90 ml of water (ultrapure) for 1 - 2 minutes by use of a blender. Pipette 1 ml of the suspension into an Eppendorf-tube or similar centrifugation tube and centrifuge for 5 minutes at maximum speed. Remove lipid layer by suction!

Take 20 μ l of the supernatant and dilute it with 10 ml of distilled water (*for this dilution step, do not use any glass ware!*).

Use 100 μ l for the acylation!

Assay characteristics were validated with fish-samples. For sausage the values (see chapter 8) may differ slightly.

5.3 Cheese

Homogenize 10 g of cheese in 90 ml of water (ultrapure) for 1 - 2 minutes by use of a blender. Pipette 1 ml of the suspension into an Eppendorf-tube or similar centrifugation device and centrifuge for 5 minutes at maximum speed. Remove lipid layer by suction!

Take 20 μ l of the supernatant and dilute it with 10 ml of distilled water (*for this dilution step, do not use any glass ware!*).

Use 100 μ l for acylation!

Assay characteristics were validated with fish samples. For cheese the values (see chapter 8) may differ slightly.

5.4 Milk

(a "precipitator" is needed for this preparation. Please ask your local supplier.)

Pipette 10 µl of milk into an Eppendorf-tube or similar centrifugation device. Add 50 µl of precipitator. Vortex mix, incubate for 5 minutes and add 2 ml of 0.1 N hydrochloric acid (HCl).

Centrifuge for 5 minutes at 3,000 x g and remove the lipid layer by suction.

Use 100 µl for the acylation!

5.5 Wine, champagne

Dilute 20 µl with 10 ml distilled water (for this dilution step, do not use any glass ware!).


Use 100 µl for the acylation!

Assay characteristics were validated with fish samples. For wine and champagne the value (see chapter 8) may differ slightly.

6. Test procedure

Allow reagents and samples to reach room temperature and mix thoroughly by gentle inversion before use. Duplicate determinations are recommended. It is recommended to number the strips of the microwell plate before usage to avoid any mix-up.

The binding of the antisera and of the enzyme conjugate and the activity of the enzyme are temperature dependent, and the absorption values may vary if a thermostat is not used. The higher the temperature, the higher the absorption values will be. Varying incubation times will have similar influences on the absorbance. The optimal temperature during the Enzyme Immunoassay is between 20 - 25 °C.

 In case of overflow, read the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to 405 nm

6.1 Preparation of reagents

Wash Buffer

Dilute the 20 ml Wash Buffer Concentrate with water (deionized, distilled, or ultra-pure) to a final volume of 1000 ml.

Storage: 1 month 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 Acylation

1. Pipette 100 µl of standards, controls and extracts into the respective wells of the Reaction Plate .
2. Add 25 µl of Acylation Reagent (refer to 6.1) to all wells.
3. Pipette 200 µl of Acylation Buffer into all wells.
4. Incubate 15 min at RT (20 – 25 °C) on a shaker (approx. 600 rpm) Alternatively without shaker: shake the plate shortly by hand and incubate for 15 min at RT.
 Take 25 µl for the ELISA

6.3 Histamine ELISA

1.	Pipette 25 µl of the acylated standards, controls and samples into the wells of the Histamine Microtiter Strips .
2.	Pipette 100 µl of the Histamine Antiserum into all wells.
3.	Incubate 30 min at RT (20 – 25 °C) on a shaker (approx. 600 rpm). Alternatively without shaker: shake the Histamine Microtiter Strips shortly by hand and incubate for 40 min at RT (20 – 25 °C).
4.	Discard or aspirate the content 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 Enzyme Conjugate into all wells.
6.	Incubate for 10 min at RT (20 – 25 °C) on a shaker (approx. 600 rpm). Alternatively without shaker: incubate for 20 min at RT (20 – 25 °C).
7.	Discard or aspirate the content 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.
8.	Pipette 100 µl of the Substrate into all wells.
9.	Incubate for 15 ± 2 min at RT (20 – 25 °C) on a shaker (approx. 600 rpm). Alternatively without shaker: incubate for 15 ± 2 min at RT (20 – 25 °C). Avoid exposure to direct sunlight!
10.	Add 100 µl of the Stop Solution to each well and shake the microtiter plate to ensure a homogeneous distribution of the solution.
11.	Read the absorbance of the solution in the wells within 10 minutes , using a microplate reader set to 450 nm (if possible, a reference wavelength between 620 nm and 650 nm is recommended).

7. Calculation of results

Standard	Concentration of the standards					
	A	B	C	D	E	F
Histamine ng/ml (ppb)	0	0.5	1.5	5	15	50
Conversion:	Histamine (ng/ml) = Histamine (µg/l) = Histamine (µg/kg) = Histamine (ppb) Histamine (ng/ml) x 9 = Histamine (nmol/l)					

The standard curve from which the concentrations of the samples can be read off, is obtained by plotting the absorbance readings (calculate the mean absorbance) measured for the standards (linear, y-axis) against the corresponding standard concentrations (logarithmic, x-axis).

Use non-linear regression for curve fitting (e.g. spline, 4- parameter, akima).

⚠ The histamine concentration in µg/l (ppb) of each sample is read from the standard curve and has to be **multiplied** by the corresponding **dilution factor**. The dilution factor depends on the sample preparation method.

Preparation method	5.1	5.2	5.3	5.4
Sample	fish meal	fresh fish, sausage, cheese	milk	wine, champagne
Dilution Factor	200,000	5,000	200	500

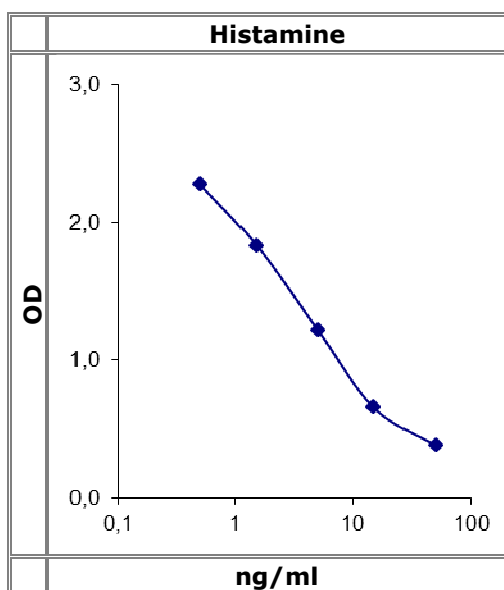
7.1 Quality control

It is recommended to use control samples according to national regulations. Use controls at both normal and pathological levels. The kit or other commercially available controls should fall within established confidence limits. The confidence limits of the kit controls are listed in the QC-Report.

7.2 Typical standard curve



Example, do not use for calculation!



8. Assay characteristics

Analytical Specificity (Cross Reactivity)	Substance	Cross Reactivity (%) Histamine
	Histamine	100
	3-Methyl-Histamine	0.1
	Tyramine	0.01
	L-Phenylalanine	< 0.001
	L-Histidine	< 0.001
	L-Tyrosine	< 0.001
	Tryptamine	< 0.001
	5-Hydroxy-Indole-Acetic Acid	< 0.001
Serotonin	< 0.001	

Analytical Sensitivity	Histamine	
	LOB	0.12 ng/ml
	LOD	0.18 ng/ml
	LOQ	0.38 ng/ml

Precision					
Inter-Assay Variation			Intra-Assay Variation		
Sample	Mean ± SD [ng/ml (ppb)]	CV (%)	Sample	Mean ± SD [ng/ml (ppb)]	CV (%)
1	4.8 ± 0.6	11.5	1	1.3 ± 0.3	19.3
2	21.5 ± 2.9	13.4	2	4.9 ± 0.7	13.9
			3	13.5 ± 1.5	11.2

Precision Recovery			
		Range (%)	Mean (%)
	Fish meal	76 - 106	92
	Mackerel	78 - 100	92
	Canned Tuna	89 - 102	95
	Fresh Tuna	88 - 97	91
	White wine	94 - 108	101



Red wine	99 - 116	109
Champagne	95 - 109	99
Milk	83 - 110	98
Sausage	85 - 104	95
Cheese	74 - 115	99

Method comparison	The histamine concentration in fish meal samples provided by IFFO, UK, was assessed using both this ELISA (x) and HPLC (y). The results of linear regression analysis yielded the following correlation characteristics: $y = 1.4x + 10$, $r = 0.9$ (n=20). The two assays show a high correlation.
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9. References/Literature

1. P.L. Rogers, W.F. Staruszkiewicz, 2000. Histamine Test Kit Comparison. Journal of Aquatic Food Product Technology 9 (2), 5-17
2. J.M. Hungerford, 2010. Scombroid poisoning: a review. Toxicon 15, 231-243
3. S. Köse, N. Kaklikkaya, S. Koral, B. Tufan, K.C. Buruk, F. Aydin, 2011. Commercial test kits and the determination of histamine in traditional (ethnic) fish products-evaluation against EU accepted HPLC method. Food Chemistry, 125 (4) 1490-149

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-  **For updated literature or any other information please contact your local supplier.**
-  **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:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Expiry date		Batch code		
	Consult instructions for use		Content		
	Caution		Catalogue number		For research use only!