Instructions for use

Histamine Food ELISA

REF **FC E-3100**

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 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).
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 antiserum binding sites. When the system is in equilibrium, free antigen and free antigen-antiserum 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. Advice on handling the test

2.1 Reliability of the test results

In order to assure a reliable evaluation of the test results it must be conducted according to the instructions included and in accordance with current rules and guidelines (e.g. GLP, RILIBÄK, etc.). Special attention must be paid to control checks for precision and correctness during the test; the results of these control checks have to be within the norm range. In case of significant discrepancies between the pre-set assay characteristics of this test and the actual results please contact the manufacturer of the test kit for further instructions.

2.2 Complaints

In case of complaints please submit to the manufacturer a written report containing all data as to how the test was conducted, the results received and a copy of the original test printout. Please contact the manufacturer to obtain a complaint form and return it completely filled in to the manufacturer.

2.3 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 incurred in transit.

2.4 Disposal

Residual substances and/or all remaining chemicals, reagents and ready for use solutions, are special refuse. The disposal is subject to the laws and regulations of the federation and the countries. About the removal of special refuse the responsible authorities or refuse disposal enterprises inform. The disposal of the kit must be made according to the national official regulations. Legal basis for the disposal of special refuse is the cycle economic- and waste law.
The appropriate safety data sheets of the individual products are available on the homepage. The safety data sheets correspond to the standard: ISO 11014-1.

2.5 Interference

Do not mix reagents and solutions from different lots. Consider different transport and storage conditions. Inappropriate handling of test samples or deviations from the test protocol can affect the results . Use no kit components beyond the expiration date. Avoid microbiological contamination of the reagents and the washing water. Consider incubation periods and wash references.

2.6 Precautions

Never pipette by mouth and avoid contact of reagents and specimens with skin. No smoking, eating or drinking in areas where samples or kit test tubes are handled. When working with kit components or samples, always wear protective gloves and wash your hand thoroughly as soon as you have finished the work. Avoid spraying of any kind. Avoid any skin contact with reagents. Use protective clothing and disposable gloves. Optimal test results are only obtained when using calibrated pipettes.

3. Storage and stability

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

4.1 Contents of the kit

BA D-0024	REAC-PLATE	**Reaction Plate**	1 x 96 wells	ready for use
BA E-0030	WASH-CONC 50x	**Wash buffer Concentrate**	1 x 20 mL	concentrate, dilute content with dist. water to a final volume of 1000 mL
BA E-0055	SUBSTRATE	**Substrate**	1 x 12 mL	ready for use, containing a solution of tetramethylbenzidine (TMB)
BA E-0080	STOP-SOLN	**Stop Solution**	1 x 12 mL	ready for use, containing 0.25 M H_2SO_4.
BA E-1031	MTP HIS	**Histamine Microtiter Strips**	1 x 96 wells	12 strips, 8 wells each, break apart, precoated
BA E-1001	STANDARD A	**Standard A**	1 x 4 mL	ready for use
BA E-1002	STANDARD B	**Standard B**	1 x 4 mL	ready for use
BA E-1003	STANDARD C	**Standard C**	1 x 4 mL	ready for use
BA E-1004	STANDARD D	**Standard D**	1 x 4 mL	ready for use
BA E-1005	STANDARD E	**Standard E**	1 x 4 mL	ready for use
BA E-1006	STANDARD F	**Standard F**	1 x 4 mL	ready for use
BA E-1051	CONTROL 1	**Control 1**	1 x 4 mL	ready for use
BA E-1052	CONTROL 2	**Control 2**	1 x 4 mL	ready for use
BA E-1210	HIS-AS	**Histamine Antiserum**	1 x 12 mL	from goat, ready for use
BA E-1211	ACYL-BUFF	**Acylation Buffer**	2 x 12 mL	ready for use
BA E-1212	ACYL-REAG	**Acylation Reagent**	2 x 1,5 ml	ready for use
BA E-1240	CONJUGATE	**Enzyme Conjugate**	1 x 12 ml	ready for use, anti-goat IgG conjugated with peroxidase

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

- Calibrated variable precision micropipettes (e.g. 10-100 µL / 100-1000 µL)
- ELISA plate reader capable of reading absorbance at 450 nm
- Absorbent material (paper towel)
- Distilled water
- Vortex mixer

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 3mm; 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 Fish meal

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 µl of the supernatant and dilute it with 20 ml of distilled water *(for this dilution step, do not use any glass ware!).* Use 100 µl for the acylation!

5.2 Fresh fish, sausage (processed, smoked or fermented meats), cheese

Homogenize 10 g of fresh fish (sausage, cheese) in 90 ml of distilled water for 1 – 2 minutes by use of a house-hold food mincer. 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 µl of the supernatant and dilute it with 10 ml of distilled water *(for this dilution step, do not use any glass ware!)*. Use 100 µl for the acylation!

5.3 Milk

(a " precipitator" is needed for this preparation. Please ask your local supplier.)
Pipette 10 µl of milk into a centrifugation tube. 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!

5.4 Wine, champagne

Dilute 20 µl with 10 ml of distilled water *(for this dilution step, do not use any glass ware!)*.
Use 100 µl for acylation!

6. Test procedure

Allow reagents and samples to reach room temperature. Duplicate determinations are recommended.

6.1 Preparation of reagents

Wash Buffer
Dilute the 20 mL Wash Buffer Concentrate with distilled water to a final volume of 1000 mL. Store the diluted Wash Buffer Concentrate (Wash Buffer) at 2 – 8 °C. Shelf life: please refer to the expiry date indicated on the kit.

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. Alternative the Acylation Reagent can be stored at room temperature (20 – 25°C) separate from the other kit components.

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 minutes** at **RT** (20-25°C) on a shaker (approx. 600 rpm) Alternative protocol 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 contents of the wells and **wash** each well **3 times** thoroughly with **300 µL Wash Buffer**. Blot dry 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 contents of the wells and **wash** each well **3 times** thoroughly with **300 µL Wash Buffer**. Blot dry 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** *at* ***RT*** *(20-25°C).* ***Avoid exposure to direct sun light!***
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** and a reference wavelength between 620 nm and 650 nm.

7. Calculation of results

	Concentration of the standards					
Standard	**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)					

The calibration 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 calibration 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 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
Bluefin 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-iuy mullet	fresh
Tuna	canned
Different species	Fish Meal
Different species	Fish paste

7.2 Quality control

It is recommended to use control samples according to state and federal regulations. Use controls at both normal and contaminated levels. The kit or other commercially available controls should fall within established confidence limits. The confidence limits of the kit controls are indicated on the QC-Report.

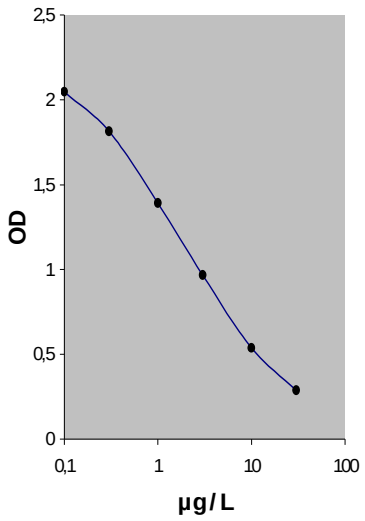
7.3 Calibration

The binding of the antisera and the enzyme conjugates and the activity of the enzyme used are temperature dependent, and the extinction values may vary if a thermostat is not used. The higher the temperature, the higher the extinction values will be. The extinction values also depend on the incubation times. 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*

7.4 Typical calibration curve

Histamine



⚠ *Example. Do not use for calculation!*

8. Assay characteristics

Analytical Specificity (Cross Reactivity)	**Substance**	**Cross Reactivity (%) Histamine**
	Histamine	100
	3-Methyl-Histamine	0.01
	Tyramine	< 0.001
	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 (Limit of Detection)	Histamine	0.15 µg/L	Mean signal (Zero-Standard) - 2SD

Precision					
Inter-Assay Variation, n = 13			**Intra-Assay Variation**, n = 39		
Sample	Mean ± SD [µg/L (ppb)]	CV (%)	Sample	Mean ± SD [µg/L (ppb)]	CV (%)
1	2.03 ± 0.16	8	1	0.6 ± 0.1	12
2	6.74 ± 0.37	5.6	2	4.6 ± 0.3	6.3

Precision			
Recovery		**Range (%)**	**Mean (%)**
	Milk	95 - 146	118
	Wine	87 - 107	95
	Fish	95 - 133	106
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.		

⚠ ***For current literature, information about clinical significance or any other information please contact your local supplier.***

Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Expiry date	LOT	Batch code	IVD	For in-vitro diagnostic use only!
	Consult instructions for use	CONT	Content	CE	CE labelled
	Caution	REF	Catalogue number	RUO	For research use only!