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Instructions for use

Histamine Food ELISA

REF IB89141

2°C 8°C

Σ 96

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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 Food 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 – 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 H_2SO_4. 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 of according to national regulations.
(18) 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.

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 kit and reagents at 2 – 8 °C until expiration date. Do not use kit and components beyond the expiry date indicated on the kit labels. Once opened, the reagents are stable for 2 months when stored at 2 – 8 °C. Once the resealable pouch of the ELISA plate has been opened, care should be taken to close it tightly again including the desiccant.

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, 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	
BA E-1031	**HIS**	**Histamine Microtiter Strips** – ready to use
Content:	1 x 96 wells (12x8) antigen precoated microwell plate in a resealable pouch with desiccant	
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	
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, white cap	

4.2 Calibration and Controls

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**	yellow	0.5	4.5	4 ml
BA E-1003	**STANDARD C**	orange	1.5	13.5	4 ml
BA E-1004	**STANDARD D**	blue	5	45	4 ml
BA E-1005	**STANDARD E**	grey	15	135	4 ml
BA E-1006	**STANDARD F**	black	50	450	4 ml
BA E-1051	**CONTROL 1**	green	Refer to QC-Report for expected value and acceptable range.		4 ml
BA E-1052	**CONTROL 2**	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

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

- Calibrated precision pipettes to dispense volumes between 10 – 300 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)
- Blender
- Scale
- Graduated cylinder
- Centrifugation devices (approx. 2 ml)
- Plastic tubes (≥ 10 ml)

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 mediterranan 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 regulations.

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 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 acylation!

D. FISHMEAL
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 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 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 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!
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 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 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 all reagents and samples to reach room temperature and mix thoroughly by gentle inversion before use. Number the microwell plates (Microtiter Strips which are removed from the frame for usage should be marked accordingly to avoid any mix-up). Duplicate determinations are recommended.
The binding of the antisera and of the enzyme conjugate and the activity of the enzyme are temperature dependent. 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 **WASH-CONC** **50X** with water to a final volume of 1000 ml.
Storage: 2 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 Acylation

1.	Pipette **100 µl** of **standards, controls** and **extracts** into the respective wells of the **REAC-PLATE**.
2.	Add **25 µl** of **ACYL-REAG** *(refer to 6.1)* to all wells.
3.	Pipette **200 µl** of **ACYL-BUFF** 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** **HIS**.
2.	Pipette **100 µl** of the **HIS-AS** 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** **HIS** 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 **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-SOLN** 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

	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] histamine [ng/ml] x 9 = histamine [nmol/l]					

The standard curve, which can be used to determine the concentration of the unknown samples, is obtained by plotting the absorbance readings (calculate the mean absorbance) of the standards (linear, y-axis) against the corresponding standard concentrations (logarithmic, x-axis) using a concentration of 0.001 ng/ml for Standard A (this alignment is mandatory because of the logarithmic presentation of the data). Use non-linear regression for curve fitting (e. g. 4-parameter, marquardt).

⚠*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:*

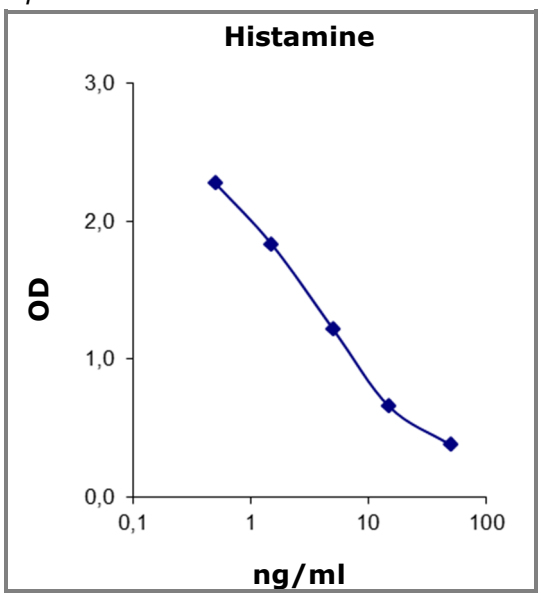
Preparation method	5.1	5.2	5.3	5.4
Sample	fish meal	fresh fish, sausage, cheese	milk	wine, champagne
Dilution Factor	200,000	5,000	200	500

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 Sensitivity	
	Histamine
Limit of Blank (LOB)	0.12 ng/ml
Limit of Detection (LOD)	0.18 ng/ml
Limit of Quantification (LOQ)	0.38 ng/ml

Analytical Specificity (Cross Reactivity)	
Substance	**Cross Reactivity [%]**
	Histamine
Histamine	100
3-Methylhistamine	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

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

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

10. 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

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:

+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		