

Instructions for use
HistaSure[™] **Fish** Rapid Test

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

HistaSure™: Histamine Screening Test

1. Intended use

The HistaSure™ assay kit is intended for the rapid screening of histamine in different scombroid fish types such as tuna, mahi mahi, sardines and for the screening of histamine in fishmeal.

2. Principle of the test

After a simple and quick water extraction step, the histamine in the sample is quantitatively derivatized into N-acylhistamine. After dilution of the N-acylated histamine in running buffer the Lateral flow device is added to the sample. The amount of immunogold labelled antibody bound to the solid phase histamine is inversely proportional to the histamine concentration in the sample.

The combination of the unique immunogold labeling technique and the highly specific immunoreagents provides a sensitive and flexible test system: Cut-offs can be adjusted quite easily depending on the requirements. If there is a need for cut-off adjustments please contact the manufacturer directly to get your customized solution.

Histamine testing in 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 have been associated with many instances of poisoning commonly referred to as "scombroid poisoning," a major health problem for consumers. Scombrototoxic fish usually contain 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.)

3. Precautions

- (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 test instructions 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 protective gloves and protective glasses where necessary.
- (4) Do not freeze the test kit or any single reagent.
- (5) The kit and single reagents should be stored dry at room temperature (18 – 25 °C / 64 – 77 °F).
- (6) For dilution or reconstitution purposes, use deionized, distilled, or ultra-pure water.
- (7) Unused Lateral Flow devices must always be stored in the desiccant container.
- (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 devices 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) Do not mix kit components with different lot numbers within a test and do not use reagents beyond the expiry date as shown on the kit labels.
- (12) For information on hazardous substances included in the kit please refer to the Safety Data Sheet. The Safety Data Sheet for this product is made available directly on the website of the manufacturer or upon request.
- (13) Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.

4. Storage and stability

The kit and single reagents should be stored dry at room temperature (18 – 25 °C / 64 – 77 °F) until expiration date. Do not use components beyond the expiry date indicated on kit labels. Unused Lateral Flow devices must always be stored in the desiccant container.

5. Content of the kit

The HistaSure™ (FC L-3200) contains materials for 24 semi-quantitative determinations of histamine.

FC L-3112	ACYL CAPS	Acylation Caps - Ready to use
Contents:	1 x 24 pieces <i>blue caps!</i>	
FC L-3331	LFA HIS	Lateral Flow Devices - Ready to use
Contents:	1 x 24 pieces	
FC L-3333	RUN-BUFF-VIAL	Running Buffer Vials - Ready to use
Contents:	1 x 24 pieces <i>red caps!</i>	
FC L-3234	ACYL-BUFF-VIAL	Acylation Buffer Vials - Ready to use
Contents:	1 x 24 pieces <i>white caps!</i>	

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

Available from LDN:

- 100 µl precision pipette (LDN catalogue # FC L-3560; 1 piece)
- pipette tips (LDN catalogue # FC L-3561; 96 pieces)

Not available from LDN:

- Grinder (mill) or house hold blender
- Graduated plastic cylinder (250 ml)
- Water (deionized, distilled, or ultra-pure)
- Scale (capable of weighing 5 – 50 grams)
- Funnel and filter paper (or alternatively a centrifuge)
- Timer
- Waterproof marker

6. Test procedure

6.1 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.

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. FISH MEAL

Mix sample until homogenous.

6.2 Histamine screening test

The following protocol will provide a **cut-off set at 50 ppm Histamine**. If there is a need for cut-off adjustments please contact the manufacturer directly to get your customized solution.

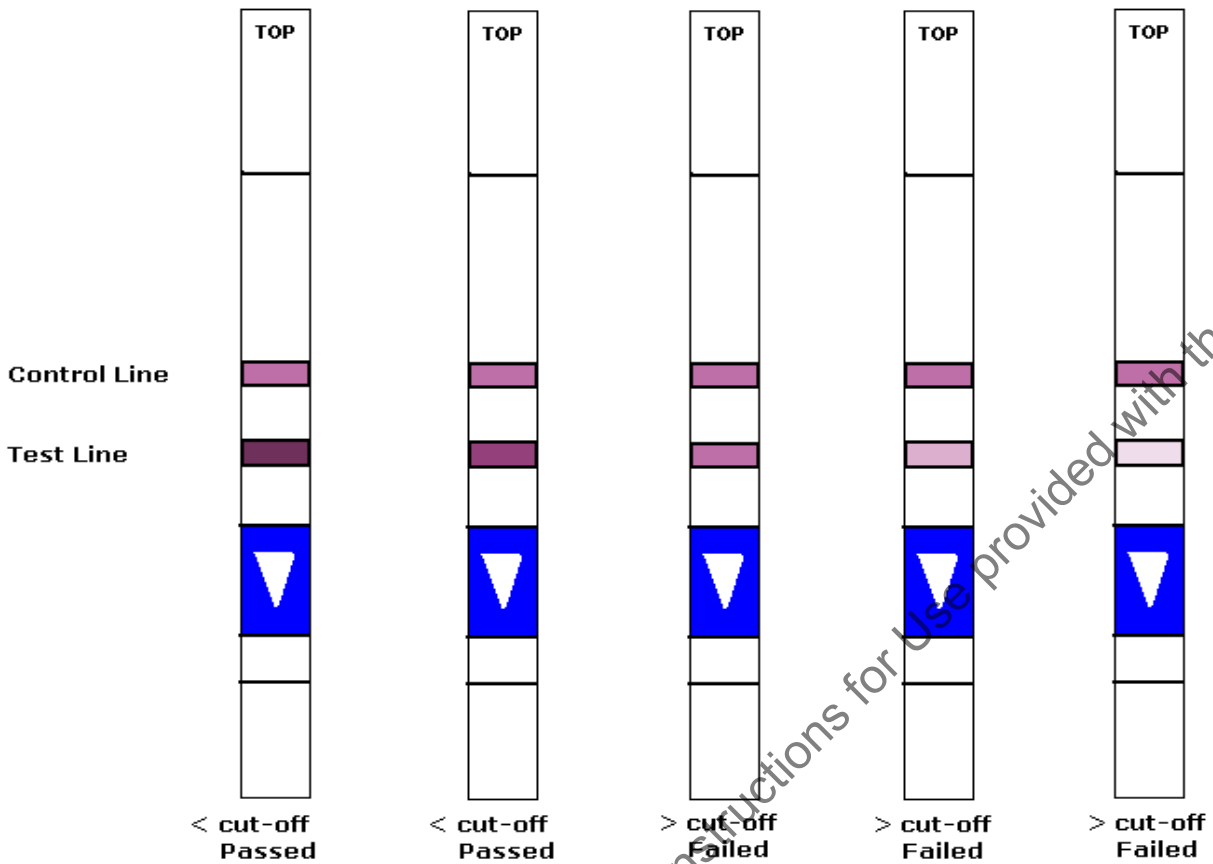
All reagents should have room temperature prior to use (room temperature = 18 – 25 °C / 64 - 77 °F).

1.	Weigh 10 g of prepared fish sample / fish meal, add 240 ml distilled water 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.
2.	Filter the homogenate through folded filter paper (alternatively an aliquot of the homogenate can be centrifuged for 5 minutes at maximum speed). <i>If a lipid layer forms remove it by suction!</i>
3.	Pipette 100 µl of the filtered homogenate into the Acylation Buffer Vials .
4.	Screw down the Acylation Buffer Vials with the Acylation Caps (FC L-3112, <i>blue caps!</i>) and mix vigorously by hand.
5.	Incubate the vials for 5 minutes at room temperature .
6.	Pipette 100 µl of the acylated samples into the Running Buffer Vials (<i>red caps!</i>). Cap the vials and mix gently.
7.	Add the Lateral Flow Device (blue part, arrow down) to the Running Buffer Vial .
8.	Incubate for 5 minutes and remove the Lateral Flow Device from the Running Buffer Vial .
9.	Read the results visually within 5 minutes .

7. Results and Interpretation

1. The control line (upper line) should be visible. This is the confirmation that the test has operated correctly. In case the control line is not visible, test is invalid and must be repeated.
2. Compare the intensity of the test line to the intensity of the control line.
3. In case the test line has higher signal intensity than the control line the sample is below the cut-off (sample has passed).
4. In case the test line has a lower signal intensity than the control line the sample is above the cut-off (sample has failed).
5. In case signal intensity of the test line and control line is equal then the sample is above the cut-off (sample has failed).

Examples of results and interpretation:



8. Application lists for different kind of fish samples

The **HistaSure™ Fish^{Rapid} Test** has been validated for the below presented fish species (other fish species are applicable, please contact the manufacturer directly).

Fish Species	Presentation
Tuna	- canned chunk light fresh/frozen yellow fin
Mahi Mahi	- fresh/frozen
Sardines	- canned in oil
Fishmeal	

9. Cut- off adjustment

The cut-off can easily be adjusted during the sample extraction step by varying the amount of distilled water in which the fish sample is homogenized:

Designated cut-off *)	Procedure
100 ppm	Homogenize 10 grams fish sample in 490 ml dist. water
50 ppm	Homogenize 10 grams fish sample in 240 ml dist. water
20 ppm	Homogenize 10 grams fish sample in 90 ml dist. water
10 ppm	Homogenize 10 grams fish sample in 40 ml dist. water

*) Any other cut-off can be derived from the examples given above.

Please note:

Cut-offs lower than 10 ppm are not recommended, because the homogenization and filtration procedures cannot be performed in an effective way.

Canned tuna and canned sardines samples were prepared according to cut-off 10, 25, 50 and 100 ppm sample preparation and spiked with histamine below, at and above the cut-off.

10. 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.

Customer Service

For customer assistance and technical support please contact

For the Americas:

Rocky Mountain Diagnostics, Inc.
Tel: 1-877-477-0039
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Training











In contrast to other available methods for the screening of Histamine in fish, the performance of the HistaSure™ is quite easy to learn and can be run by each quality control personnel.

Nonetheless LDN offers training on this product and all other LDN test kits in its own laboratories or on-site. Please contact us to arrange a testified training.

⚠ **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!