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Instructions for use
HistaSure™

REF

FC L-3200



RUO

For Research use only-
Not for use in diagnostic
procedures

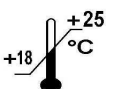


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1. **Intended use and principle of the test**

The assay kit provides materials for the semi-quantitative determination of derivatized histamine in fish^{*)} and wine (red wine, white wine, champagne).

The derivatization of Histamine is part of the preparation of the samples. By using the acylation reagent, histamine is quantitatively derivatized into N-acylhistamine. The amount of fluorescence labeled antibody bound to the solid phase histamine is inversely proportional to the histamine concentration of the sample.

The HistaSure™ assay uses the unique *FLORIDA* Technology (Fluorescence Labeled Optical-Read Immuno Dipstick Assay) which is designed to determine Histamine with highest precision in different kinds of fish samples. Even under difficult light conditions or in complete darkness the test signals can be read quite easily by visual evaluation. In contrast to gold and latex beads used in traditional rapid immunoassays HistaSure™ uses a fluorescence dye to label the antibody.

The combination of *FLORIDA* and the highly specific immunoreagents shows sensitivity as high as 1 ppm and allows for the flexible adjustment of the cut-off:

- For fish samples the cut-off of the HistaSure™ kit is set to 50 and 15 ppm, but depending on the requirements, dipsticks with cut-offs as low as 10 ppm can be easily produced and adopted to different procedures. In such cases please contact the manufacturer directly to get your customized solution.
- For wine samples the cut-off of the assay can easily be adapted in a range of 2 ppm and 100 ppm with the dipsticks and reagents included in this kit just by predilution of the samples with water.

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.)

*) : Using different sample pre-treatment schemes the HistaSure™ may be used with fresh fish, canned fish, salted fish, fish in oil, and fish meal.

2. **Precautions**

- Follow the test instructions and use the indicated incubation times. Deviations from the protocol may lead to inaccurate results.
- Do not mix reagents and solutions from different lots.
- Do not use kit components beyond the expiry dates.
- To avoid any cross-contamination clean pipette tips have to be used for each sample.
- Consider the different storage conditions of the Running Buffer Tubes (at 2-8°C) and of the other kit components (at room temperature: 20-25°C).
- Allow the Running Buffer Tubes to reach room temperature prior to use.
- Unused dipsticks (FC L-3231) must always be stored in the resealable pouch with a desiccant pack. The pouch must be resealed to protect from moisture.
- Unused Histamine Antiserum Microtiter Wells (FC L-3232) must always be stored in the resealable pouch with a desiccant pack. The pouch must be resealed to protect from moisture.

3. **Storage and stability**

Except of the Running Buffer Tubes, the reagents should be stored dry at room temperature (20-25°C). The Running Buffer Tubes should be stored at 2-8°C.

Stability of the reagents: unopened at 2-8°C until expiration date indicated on the labels.

4. Contents of the kit

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

FC L-3212	ACYL-REAG	Acylation Reagent	1 x 3 mL	ready for use
FC L-3231	24 HIS-DIPSTICK	Histamine Dipstick	1 x 24	ready for use
FC L-3232	24 HIS	Histamine Antiserum Microtiter Wells	1 x 24	ready for use
FC L-3233	RUN-BUFF-TUBES	Running Buffer Tubes	1 x 24 x 1.5 mL	ready for use, <i>white caps!</i>
FC L-3234	ACYL-BUFF-TUBES	Acylation Buffer Tubes	1 x 24 x 0.6 mL	ready for use
FC L-3250	CONTROL	Histamine Dipstick Control	1 x 1	ready for use

5. Additional materials and equipment required but not provided with the kit

The following items are needed for wine and fish samples and can be ordered separately or as a complete add-on (equipment kit: catalogue no. FC L-3500) at LDN:

Product	Cat. No.	Quantity
100 µl precision pipette	FC L-3560	1
Pipetting tips	FC L-3561	96
LED blue light source	FC L-3565	1
Tube rack	FC L-3575	1
Orange colored lab eyewear protection glasses	FC L-3570	1

In addition the following items (not available from LDN) are needed for **fish samples**:

- grinder (mill) or house hold mincer
- graduated plastic or glass cylinder (250 ml)
- Distilled water
- pair of scissors
- funnel and filter (or alternatively a centrifuge)

For **wine samples** only distilled water and a pair of scissors are needed additionally.

6. Test procedures

6A. Test procedure for fish samples

The following protocols are suitable for fish samples (fresh fish, canned fish, and salted fish). For fish meal and fish in oil samples please contact the manufacturer directly to obtain a specific protocol.

Allow all reagents (Running Buffer Tubes) to reach room temperature prior to use.

6A.1 Sample preparation and acylation for samples with the cut-off set to 50 ppm

1. Weigh out a certain amount of fish (in gram), add 24 volumes of water (in ml) and homogenize for 1-2 minutes (e.g. 10g of fish in 240 ml H₂O)*
2. Filter the homogenate through folded filter paper (alternatively an aliquot of the homogenate can be centrifuged for 5 minutes at maximum speed). Remove lipid layer by suction!
3. Pipet 100 µl of the filtered homogenate (alternatively 100 µl of the supernatant) into the Acylation Buffer Tubes .
4. Add 100 µl of Acylation Reagent to each Acylation Buffer Tube (<i>the colour changes to pink and indicates that all pipetting steps so far have been performed accurately</i>), cap the tubes and mix gently. Incubate the tubes for 5 minutes at room temperature (after this acylation step, the samples can be stored in the Acylation Buffer Tubes at 2-8°C for 2 week or at -18°C for 1 year).
5. Pipet 100 µl of the acylated samples into the Running Buffer Tubes (white caps!). Cap the tubes and mix gently.
6. Continue with 6C. Immuno Dipstick Assay on page 6

*) The ratio of fish meat (weight) and water (volume) has to be exactly 1:25. The volume of water needed can be calculated according to: mL water needed = (gram fish meat: 10) x 240.

6A.2 Sample preparation and acylation for samples with the cut-off set to 15 ppm

1.	Weigh out a certain amount of fish (in gram), add 6.5 volumes of water (in ml) and homogenize for 1-2 minutes (e.g. 10g of fish in 65 ml H₂O *)
2.	Filter the homogenate through folded filter paper (alternatively an aliquot of the homogenate can be centrifuged for 5 minutes at maximum speed). Remove lipid layer by suction!
3.	Pipet 100 µl of the filtered homogenate (alternatively 100 µl of the supernatant) into the Acylation Buffer Tubes .
4.	Add 100 µl of Acylation Reagent to each Acylation Buffer Tube (<i>the colour changes to pink and indicates that all pipetting steps so far have been performed accurately</i>), cap the tubes and mix gently. Incubate the tubes for 5 minutes at room temperature (after this acylation step, the samples can be stored in the Acylation Buffer Tubes at 2-8°C for 2 week or at -18°C for 1 year).
5.	Pipet 100 µl of the acylated samples into the Running Buffer Tubes (white caps!). Cap the tubes and mix gently.
6.	Continue with 6C. Immuno Dipstick Assay on page 6

*) The ratio of fish meat (weight) and water (volume) has to be exactly **1:7.5**. The volume of water needed can be calculated according to: mL water needed = (gram fish meat: 10) x 65.

Quantitative determination of Histamine

For the quantitative determination of Histamine with the **Histamine Food ELISA** (BA 10-3100) the acylated samples (please refer to point 4. of the sample preparation) only have to be diluted 1:25 with distilled water. The diluted samples can be used directly for the **Histamine Food ELISA**.

6B. Test procedure for wine samples

The following protocol is suitable for wine samples (red wine, white wine, champagne). The cut-off of the assay is adjusted by using different sample dilutions (the samples have to be diluted with distilled water):

Cut-off	Sample dilution	Example
2 ppm	Undiluted sample	100 µl sample
4 ppm	1:2	100 µl sample + 100 µl dist. water
8 ppm	1:4	100 µl sample + 300 µl dist. water
16 ppm	1:8	100 µl sample + 700 µl dist. water
...

After diluting the samples with distilled water just ensure a homogenous solution by shaking it manually for a short while.

Allow all reagents – especially the Running Buffer Tubes - to reach room temperature prior to use.

6B.1 Sample preparation and acylation

1.	Pipet 100 µl of the wine sample into the Acylation Buffer Tubes .
2.	Add 100 µl of Acylation Reagent to each Acylation Buffer Tube (<i>the colour change to pink indicates that all pipetting steps so far have been performed accurately</i>), cap the tubes and mix gently. Incubate the tubes for 5 minutes at room temperature (after this acylation step, the samples can be stored in the Acylation Buffer Tubes at 2-8°C for 2 week or at -18°C for 1 year).
3.	Pipet 100 µl of the acylated samples into the Running Buffer Tubes (white caps!). Cap the tubes and mix gently.
4.	Continue with 6.2 Immuno Dipstick Assay on page 6

6C. Immuno Dipstick Assay

1.	Select the number of required Histamine Antiserum Microtiter Wells for the assay and fix them in the strip holder. The remaining unused wells must be placed in the resealable pouch with a desiccant pack. The pouch must be resealed to protect from moisture.
2.	Transfer 100 µl of the samples from the Running Buffer Tubes into the corresponding Histamine Antiserum Microtiter Wells . Mix the samples with the antiserum by pipetting it up and down 5 times (foaming has no negative influence on the assay performance).
3.	Incubate for 5 minutes (increased incubation times for up to 10 minutes have no negative influence on the assay performance).
4.	Place the Histamine Dipsticks (blue area, arrow pointing down) onto the bottom of the Histamine Antiserum Microtiter Wells .
5.	Incubate for 5 minutes.
6.	Remove the dipstick from the wells and cut off the blue area with a pair of scissors.
7.	Before inspecting the sample dipsticks, the LED blue light lamp has to be controlled by use of the Histamine Dipstick Control (FC L-3050): by illuminating the Histamine Dipstick Control with the LED blue light lamp two bands should be visible. If not, replace the batteries of the LED blue light lamp.
8.	Put on the orange glasses and inspect the sample dipsticks visually through illumination with the LED blue light lamp (the distance of the LED blue light lamp to the dipstick should be 1-2 cm).

7. Results and Interpretation

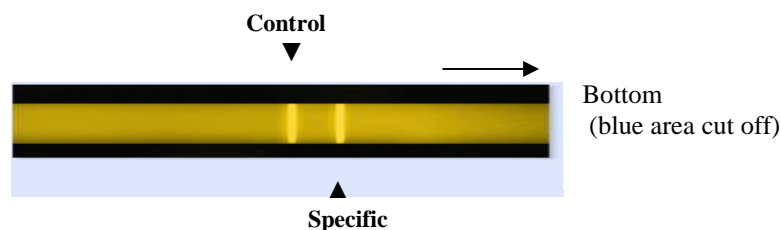
The visual inspection of the dipsticks with the LED blue light lamp can give the following results: 2 lines or 1 line*).

These results have to be interpreted the following way:

2 lines: The histamine concentration of the sample is **below** the effective cut-off; the sample has **passed** (please refer to Figure 1).

1 line: Only the control line is visible. The histamine concentration of the sample is **above** the effective cut-off; the sample has **failed** and further investigations have to be performed (e.g. quantification of Histamine with a highly-specific ELISA such as BA 10-3100. In that case the acylated samples - please refer to point 4. of the sample preparation - only have to be diluted 1:25 with distilled water. The diluted samples can be used directly for the Histamine Food ELISA).

Figure 1: Typical example for a fish sample showing 2 lines (cut-off 50 ppm; the sample has passed)



*) The Histamine Dipstick Control can be used to allocate the lines: the lower line corresponds to the specific line, the upper to the control line (please refer to Figure 1). The main proof, that the assay worked well is, that the control line is visible. If no line is visible please check the functionality of the LED blue light lamp first. If the lamp is working well, the performance of the assay was incorrect and the problem has to be solved by trouble-shooting. In that case the sample has to be re-assayed.

8. Literature

- DEVELOPMENT OF A NEW LATERAL FLOW IMMUNOASSAY FOR THE DETERMINATION OF HISTAMINE IN FISH (HISTASURE™).
3rd International Symposium on RECENT ADVANCES IN FOOD ANALYSIS; November 7 – 9, 2007, Prague, Czech Republic
Essy Booltink¹, Martijn van Faassen^{2*}, Johannes Bonenberger³, Bernhard Manz⁴
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³ Cibitest, Neu Ulm, Germany

9. 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.

Customer Service

For customer assistance and technical support please contact

For the Americas:

Rocky Mountain Diagnostics, Inc.
Tel: 1-877-477-0039
Fax: 1-719-477-0307
e-mail: info@rmdiagnosics.com

World-Wide:











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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.

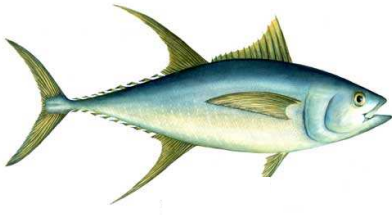
Nonetheless LDN offers training sessions in its own laboratories or on-site. Please contact us to arrange a testified demonstration.

Used symbols:

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

Flow Chart

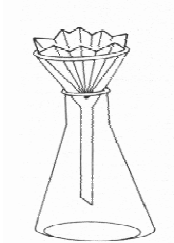
HistaSure™ Fish Sample
(cut-off: 50 ppm)



10g of fish are needed



Add 240 ml of water



Filter or



Centrifuge



Extract



Pipet 100 µl extract
into the prefilled
Acylation Buffer Tubes



Acylation reagent



Pipet 100 µl
Acylation Reagent
into the tube



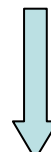
Acylation time



≥ 5 min



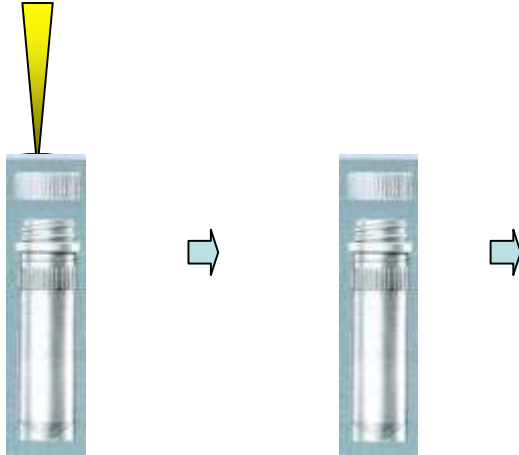
Close and
mix
by inversion



**Continued on
next page...**



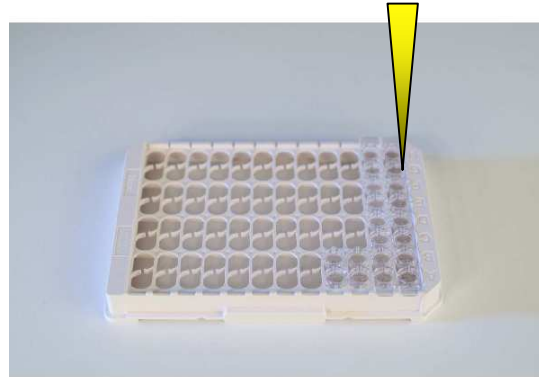
Acylated fish extract



Pipet 100 µl of the **acylated extract** into the prefilled Running Buffer Tubes (white caps!)

Close and mix by inversion

Diluted acylated fish extract



Pipet 100 µl of the **diluted** acylated extract in microtiter well, mix by pipetting up and down (5x) and wait for

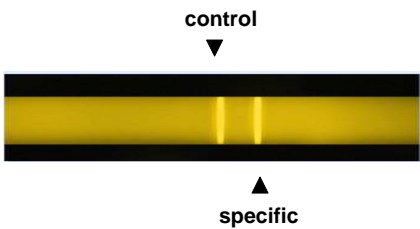


= 5 min

Use blue light LED to visualize results

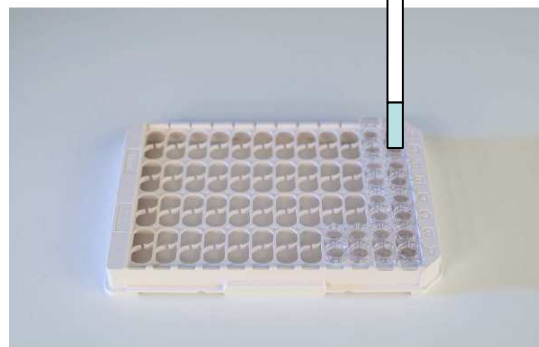


This is what you will see



Visual interpretation:

2 bands = histamine < 50 ppm
1 band = histamine > 50 ppm



Put HistaSure dipstick (blue zone down) in microtiter well and wait for



= 5 min