

Instructions for use

HistaSure™

Fluorescence Labelled Optical-Read Immuno Dipstick Assay
for the determination of Histamine
in fresh fish, canned fish, salted fish and fish meal

REF BA 50-3000



RUO

1. Intended use and Principle of the test

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.)

The HistaSure™ assay uses the unique *FLORIDA* Technology (Fluorescence Labelled 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 a sensitivity as high as 5 ppm and allows for the flexible adjustment of the cut-off. The cut-off of the HistaSure™ is set to 50 ppm but depending on the requirements, dipsticks with cut-offs as low as 5 ppm can be easily produced and adopted to different procedures. In such cases please contact the manufacturer directly to get your customized solution.

The assay kit provides materials for the semi-quantitative determination of derivatized histamine in fish extracts. The derivatization is part of the preparation of the samples. By using the acylation reagent, histamine is quantitatively derivatized into N-acylhistamine. The amount of fluorescence labelled antibody bound to the solid phase histamine is inversely proportional to the histamine concentration of the sample.

Using different sample pre-treatment schemes the HistaSure™ may be used with fresh fish, canned fish, salted fish 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.

3. Storage and stability

Except of the Running Buffer Tubes the reagents should be stored dry at room temperature (20-25°C) until expiration date. The Running Buffer Tubes should be stored at 2-8°C until expiration date. Do not use components beyond the expiration date indicated on the kit labels.

4. Contents of the kit

The HistaSure™ (BA 50-3000) contains materials for 24 semi-quantitative determinations of histamine. This kit is manufactured by: **Labor Diagnostika Nord GmbH & Co. KG, Nordhorn, Germany.**

BA 50-3012	ACYL-REAG	Acylation Reagent	1 x 3 mL	ready for use
BA 50-3031	24 HIS-DIPSTICK	Histamine Dipstick	1 x 24	ready for use
BA 50-3032	24 HIS	Histamine Antiserum Microtiter Wells	1 x 24	ready for use
BA 50-3033	RUN-BUFF-TUBES	Running Buffer Tubes	1 x 24 x 1.5 mL	ready for use, white caps!
BA 50-3034	ACYL-BUFF-TUBES	Acylation Buffer Tubes	1 x 24 x 0.6 mL	ready for use
BA 50-3050	CONTROL	Histamine Dipstick Control	1 x 1	ready for use

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

Available from LDN:

Product	Cat. No.	Quantity
100 µl precision pipette	BA 50-3060	1
Yellow pipetting tips	BA 50-3061	96
LED Blue light source	BA 50-3065	1
Tube Rack	BA 50-3075	1
Orange colored lab eyewear protection glasses	BA 50-3070	1

Not available from LDN:

- 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)

6. Test procedure

The following protocol is suitable for fresh fish, canned fish and salted fish. For fish meal please contact the manufacturer directly to obtain a specific protocol.

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

6.1 Sample preparation and acylation

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.

*) 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.

6.2 Immuno Dipstick Assay

1.	Fix the needed amount of Histamine Antiserum Microtiter Wells in the strip holder (Histamine Antiserum Microtiter Wells which are not needed should be stored in the foil with desiccant).
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 (BA 50-3050): 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

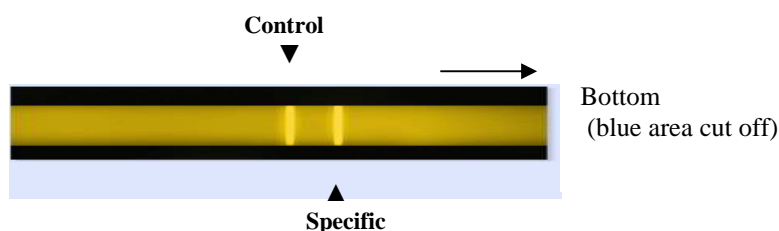
The visual inspection of the dipsticks with the LED blue light lamp can give the following results:

2 lines, 1 line or no line at all. 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). First one should check if the control line exists: this is the main proof that the assay worked well.

The results have to be interpreted the following way:

- 2 lines:** the histamine concentration of the sample is below 50 ppm; The presence of Histamine in the sample is not noticeable.
- 1 line:** *Control line visible:* the histamine concentration of the sample is above 50 ppm; the sample contains noticeable histamine and further investigations have to be performed (e.g. quantification of Histamine with a highly-specific ELISA such as BA 10-3100).
Specific line visible: this is an invalid result and has to be clarified by trouble shooting
- No line:** this is an invalid result and has to be clarified by trouble shooting (first, please check the functionality of the LED blue light lamp)

Figure 1: Typical example showing 2 lines (Histamine < 50 ppm)



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
^{1 2 4} Labor Diagnostika Nord, Nordhorn, Germany
³ Cibitest, Neu Ulm, Germany

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 call: +49 5921 8197 0 or +49 5921 8197 131 or e-mail us: support@ldn.de

Training

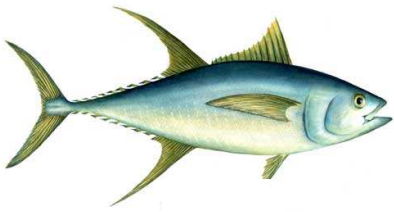
In contrast to other available methods for the screening of Histamine in fish, the performance of the HistaSure™ is quite easy to learn and can be run by each quality control personnel.

None the less 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
	For in-vitro diagnostic use only!		Content		Consult instructions for use
	For research use only!		Caution		

Flow Chart **HistaSure™**



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



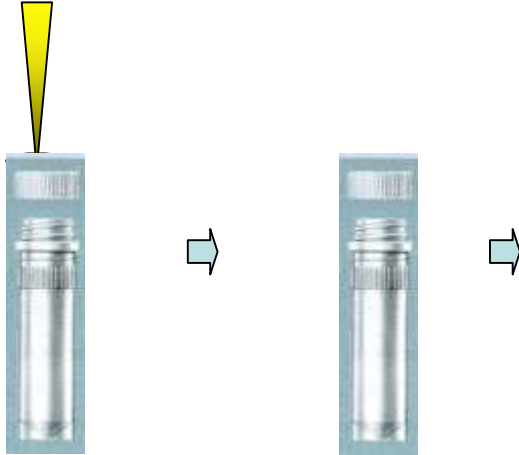
Close and
mix
by inversion



**Continued on
next page...**



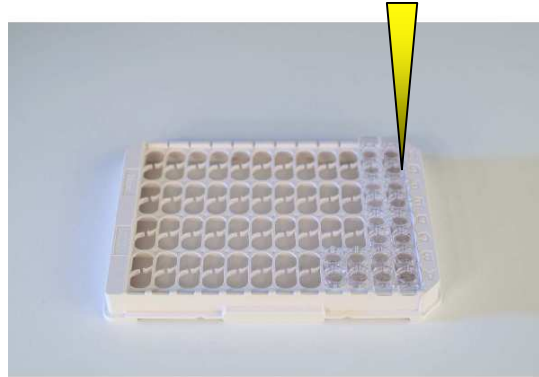
Acylated fish extract



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

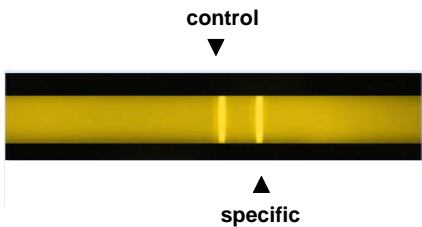


= 5 min

Use blue light LED to visualize results

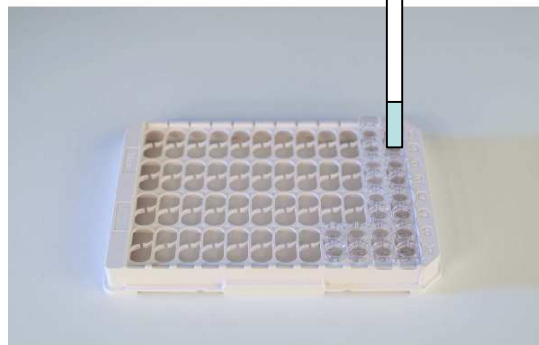


This is what you will see



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