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## Instructions for use

# HisQuick Histamin Rapid Test

**REF**



**RUO**

For Research use only -  
Not for use in diagnostic  
procedures

**LDN-FC P-3000**

## HisQuick™ colorimetric assay

### 1. **Principle of the test**

The **HisQuick™** Histamine Rapid Test is a colorimetric assay for the quantitative determination of Histamine in fish meal.














The test kit contains enough reagents to determine 48 fish meal samples.

First, Histamine is extracted from the sample and then bound to an ion-exchanger column to separate it from impurities. Next, the Histamine is derivatized and the resulting dye is determined at 450 nm. The optical density of the dye is directly proportional to the concentration of the Histamine in the sample.

### 2. **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.

### 3. **Contents of the kit**

<b>BA 20-3032</b>	 96	<b>Microtiter Plate</b>	1 x 96 wells	12 strips, 8 wells each, break-apart
<b>BA 20-3001</b>	 STANDARD A	<b>Standard A</b>	1 x 1 mL	ready for use
<b>BA 20-3002</b>	 STANDARD B	<b>Standard B</b>	1 x 1 mL	ready for use
<b>BA 20-3003</b>	 STANDARD C	<b>Standard C</b>	1 x 1 mL	ready for use
<b>BA 20-3004</b>	 STANDARD D	<b>Standard D</b>	1 x 1 mL	ready for use
<b>BA 20-3005</b>	 STANDARD E	<b>Standard E</b>	1 x 1 mL	ready for use
<b>BA 20-3006</b>	 STANDARD F	<b>Standard F</b>	1 x 1 mL	ready for use
<b>BA 20-3030</b>	 WASH-CONC 	<b>Wash Buffer Concentrate</b>	1 x 50 mL	Concentrate. Dilute content with distilled water to a final volume of 500 mL
<b>BA 20-3041</b>	 ELUTION-BUFF	<b>Elution Buffer</b>	1 x 55 mL	ready for use
<b>BA 20-3042</b>	 ION-EXCHANGER	<b>Ion Exchanger Set</b>	6 x 8	extraction columns and sample tubes, ready for use
<b>BA 20-3043</b>	 COLOR-REAG 1	<b>Color Reagent 1</b>	1 x 6 mL	ready for use
<b>BA 20-3044</b>	 COLOR-REAG 2	<b>Color Reagent 2</b>	1 x 6 mL	ready for use

### 4. **Additional materials required but not provided with the kit**

#### **Equipment:**

- microtiter plate or strip photometer (450 nm)
- grinder (mill)
- graduated cylinder: 100 ml plastic or glass
- glassware for preparing sample extract
- optional: shaker
- filter paper: Whatman No. 1 or equivalent
- test tubes
- Calibrated variable precision micropipettes (e.g. 10-100 µL / 100-1,000µL)

#### **Reagents:**

- isopropanol
- distilled or deionized water
- 70% isopropanol solution: for example, mix 70 ml isopropanol with 30 ml distilled water.

If you don't want to prepare the extraction solution by yourself, please contact your local supplier.

### 5. **Sample collection and storage**

The samples should be stored in a cool place.

A representative sample (according to accepted sampling techniques) should be grounded and thoroughly mixed prior to the extraction procedure.

## 6. Test procedure

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

### 6.1 Preparation of reagents


#### Wash Buffer

Dilute 50 ml of the Wash Buffer Concentrate with distilled water to a final volume of 500 ml. Store the diluted Wash Buffer Concentrate (Wash Buffer) at 2 – 8 °C.

#### Extraction columns (Ion Exchanger Set)

To avoid desiccation please open only as much extraction columns as you actually need! Once opened the extraction columns have to be stored in a refrigerator or at least under humid conditions!

Displace lock from the top stopper of the column first, and then remove the bottom stopper to avoid splashing of the gel matrix

 *The sample and the Wash Buffer should be applied carefully on top of the column to avoid the swirling of the gel (this would create inhomogeneous gel densities).*


#### Color reagent solution

Mix the Color Reagent 1 and 2 at a one to one ratio (this solution is ready for use and is stable for 10 hours).

### 6.2 Extraction

	Add <b>2 g</b> of the grounded sample to a suitable container together with <b>20 mL of 70% isopropanol solution</b> *)
<b>2.</b>	<b>Shake</b> vigorously for four minutes (manually or with a shaker)
<b>3.</b>	<b>Filter</b> the extract through a paper filter (e.g. Whatman No.1 filter) and <b>centrifuge</b> for <b>2 minutes</b> (or just let the solids settle down for a few minutes). <i>The <b>supernatant</b> is applied to the extraction column.</i>
	*) Other sample sizes may be used, but the volume of the 70% isopropanol solution has to be adapted accordingly. (e.g.: 20 g in 200 ml of 70% isopropanol solution)

### 6.3 Clean up

	<b>Wash</b> each <b>extraction column</b> with <b>2 mL of Wash Buffer</b> (refer to 6.1) prior to use!
<b>2.</b>	Add <b>200 µL</b> of the <b>supernatant</b> onto the <b>extraction columns</b> and allow the extract to surge into the extraction columns.
<b>3.</b>	<b>Wash</b> the <b>extraction columns</b> with <b>1 ml 70% isopropanol solution</b> .
<b>4.</b>	<b>Wash</b> each extraction column <b>2 times</b> with <b>3 mL Wash Buffer</b> (refer to 6.1).
<b>5.</b>	<b>Discard</b> the passed through Wash Buffer and use the <b>sample tubes</b> of the <b>Ion Exchanger Set</b> to collect the <b>eluates</b> .
<b>6.</b>	Pipette <b>0.5 ml Elution Buffer</b> carefully onto extraction columns, allow to surge into the extraction columns and pipette again <b>0.5 ml of Elution Buffer</b> into the extraction columns.
<b>7.</b>	 <b>Collect both eluates in the same sample-tube and vortex-mix the eluates!</b>
<b>8.</b>	Use <b>200 µl</b> for the subsequent Histamine measurement!

### 6.4. Histamine measurement

<b>1.</b>	Pipette <b>50 µl</b> each of the <b>Standard A -F</b> into the <b>Microtiter Plate</b> .
<b>2.</b>	Pipette <b>150 µl</b> of <b>Elution Buffer</b> to <b>each standard well</b> .
<b>3.</b>	Pipette <b>200 µl</b> each of the <b>extracted samples</b> into the <b>Microtiter Plate</b> .
<b>4.</b>	Add <b>50 µl</b> of the <b>color reagent solution</b> (refer to 6.1) to each well and mix.
<b>5.</b>	After 2 minutes <b>read</b> the absorbance of the solution in the wells using a microplate reader set to <b>450 nm</b> .

 *The signal is stable for at least 8 hours. A visual evaluation without reader is also possible (semi-quantitative method).*

## 7. Calculation of results

	Concentration of the standards					
Standard	A	B	C	D	E	F
Histamine, ppm (mg/kg)	0	125	250	500	1 000	2 000
Conversion:	Histamine (mg/L) = Histamine (mg/kg) = Histamine (ppm)					

The calibration curve is obtained by plotting the absorbance readings (calculate the mean absorbance) of the standards (linear, y-axis) against the corresponding standard concentrations in ppm (linear, x-axis)

The histamine concentration of the samples (in ppm) can be read directly from the calibration curve.

## 8. Assay characteristics

### 8.1 Cross reactivity

The cross-reactivity has been measured against various compounds. The percent cross reactivity is expressed as the ratio of histamine concentration to the concentration of the reacting compound at 50 % of colour reaction under assay conditions:

Substance	Cross Reactivity (%)
Histamine	100
L-Histidine	< 1
Tyramine	<1
L-Tyrosine	<1
L-Phenylalanine	not detectable (n.d.)
L-Tryptophan	n.d.
Non-aromatic amino acids	n.d.
Tryptamine	n.d.
Serotonin	n.d.

### 8.2 Correlation HPLC versus HisQuick™

$$r = 0,98$$

$$y^{\text{HPLC}} = 0.92x^{\text{HisQuick}} + 83$$

### 8.3 Sensitivity

The mean lower detection limit for histamine in fish meal is **125 mg/kg** (ppm) for fish meal.

## 9. Advice on handling the test

### 9.1 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 (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.

### 9.2 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.

### 9.3 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.

#### 9.4 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.

#### 9.5 Interference

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

#### 9.6 Precautions












Observe the incubation periods and washing instructions. 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. All steps have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes. Sodium azide could react with lead and copper tubes and may form highly explosive metal azide. When clearing up, rinse thoroughly with large volumes of water to prevent such formation.

All reagents of this testkit which contain human or animal serum or plasma have been tested and confirmed negative for HIV I/II, HbsAg and HCV by FDA approved procedures.

All reagents, however, should be treated as potential biohazards in use and for disposal.

 **Actual literature, information about clinical significance or any other information about the test are available on the homepage or contact the manufacturer directly.**

#### 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		

Li StarFish distribuisce: