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Product Manual

AgraQuant® Histamine Rapid ELISA test kit
Article number 10002018

Intended use
The AgraQuant® Histamine Rapid ELISA test kit is an immunoassay designed for the quantitative analysis of histamine in fish samples. Samples can vary from fresh to canned and salted fish, from fish meal to fish in oil. This product is intended for laboratory.

Performance characteristics

Limit of detection (LOD): 1 ppm (1 mg/kg) histamine

Limit of quantification (LOQ): 3 ppm (3 mg/kg) histamine

Range of quantification: 3 – 300 ppm (3 - 300 mg/kg) histamine

Plate format: 48 wells

Assay time: sample preparation – 15-25 minutes (approx.)
total incubation time – 20 minutes

About Histamine
Histamine is a product of decomposition of histidine caused by the growth of certain bacteria in seafood. Therefore, histamine testing in fresh fish is a possible control strategy that can be used by seafood processors in their HACCP program. Fish meal produced from materials which have been allowed to degrade prior to being processed may contain high levels of histamine and can thus be toxic. Poisoning caused be fish containing high levels of histamine is commonly referred to as “scombroid poisoning”. 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 30ppm indicates significant deterioration, and 50 ppm is considered to be evidence of definite decomposition.

Product information

About the ELISA test kit

The AgraQuant® Histamine Rapid test kit is a competitive enzyme-linked immunosorbent assay (ELISA) used for the quantification of histamine in fish samples. This product is a very sensitive detection system and utilizes highly purified antibodies raised against histamine for the quantification of traces of histamine in a variety of fish products. With AgraQuant® Histamine Rapid, fresh, salted and canned fish, as well as fish meal and fish in oil can be tested for the presence of histamine.

Storage information

Upon receipt, immediately transfer the AgraQuant® Histamine Rapid to refrigerated storage and keep it at 2-8°C (35-46°F) when not in use. Do not freeze. The Acylation Reagent can be stored at room temperature (20-25°C) separate from the other kit components. Do not use the kit beyond the expiration date indicated on the package.

Content of the kit

The AgraQuant® Histamine Rapid ELISA test kit contains the following items:

- 1 master block of 48 non-coated microwells in a zip-lock bag
- 48 histamine-coated microwells (6 eight-well strips) in a microwell holder sealed in a foil pouch
- 8 vials of ready-to-use histamine controls (0, 3, 10, 20, 30, 50, 100 and 300 ppm)
- 2 bottles of 50 mL of acylation buffer
- 1 bottle of 3 mL of acylation reagent
- 1 bottle of 20 mL of 50X concentrated wash buffer
- 1 bottle of 6 mL of histamine-antiserum conjugate
- 1 bottle of 12 mL of substrate solution
- 1 bottle of 12 mL of stop solution
- 1 piece of plate sealing tape

Materials required but not included

Extraction/Acylation Procedure:

- Blender or homogenizer
- Analytical balance
- Graduated cylinder, 250 mL
- Distilled or deionized water
- Containers for sample preparation
- Shaker (shaking amplitude 3 mm, approx. 600 rpm)
- Centrifuge or micro-centrifuge and centrifuge tubes

Assay Procedure:

- Calibrated 8-channel and single-channel pipettes with 100 µL and 1000 µL disposable plastic tips
- Timer
- Plate washer or wash bottle
- Absorbent paper towels
- 3 reagent boats for use as reagent containers for an 8-channel pipette
- Microwell reader with 450 nm filter

ELISA kit – Assay principle

Agra Quant® Histamine Rapid ELISA kits are competitive ELISA test kits for the quantitative determination of derivatized histamine in fish extracts. The derivatization is part of the sample preparation. The acylation reagent quantitatively derivatizes histamine into N-acyl-histamine. Histamine is pre-coated onto each well of the microtiter plate that comes with the kit. Upon addition of the sample solution to the well, free acylated histamine and solid-phase-coated histamine compete for a fixed number of antiserum binding sites of histamine antiserum conjugate. When the system is in equilibrium, free antigen and free antigen-antiserum conjugate are removed by the washing step. The substrate solution is added, which results in color development. The intensity of the color developed is inversely proportional to the concentration of histamine present in the samples and in the controls. A stop solution is then added, which changes the color from blue to yellow. The absorbance of each well is then measured at 450 nm. The measurement must take place within 10 minutes after adding the stop solution. To analyze the results, please refer to *Results analysis* at the end of this product manual.

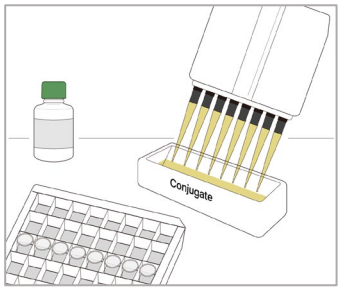
Protocol at a glance

The following section gives only an overview of the ELISA procedure. Before performing the assay, carefully read through this product manual.

1

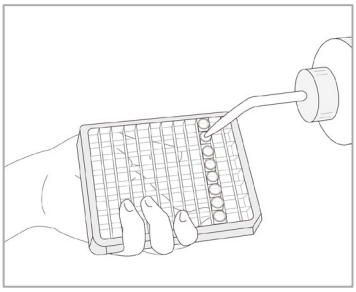
Dispense **acylated samples** and **controls** into the histamine-coated wells.

2



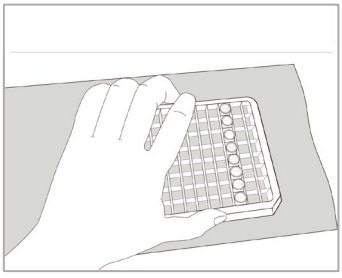
Pipette **histamine antiserum conjugate** into each well. **Incubate for 10 min.**

3



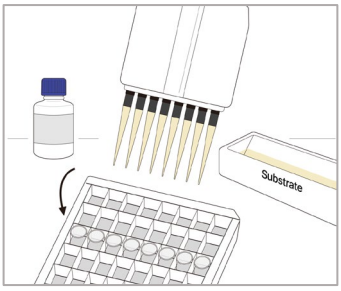
Carefully empty the wells and wash **3 times** with diluted wash buffer.

4



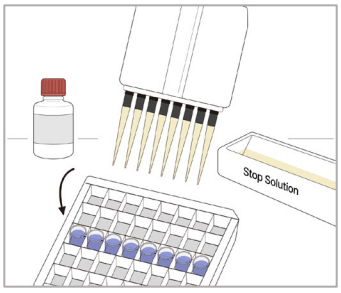
Tap the microwell strips on towels to remove all residual buffer.

5



Pipette the **substrate solution** to each well. **Incubate for 10 min** on a shaker.

6



Pipette the **stop solution** to each well. Read the absorbance of each well at **450 nm.**

7. Read the absorbance of each well at 450 nm with a microwell (ELISA plate) reader. Calculate results according to the *Results Analysis* section.

Reagent and sample preparation

Buffer preparation

Wash buffer:
Dilute the concentrated wash buffer (50X) 1:50 with distilled water (add 20 mL of concentrated wash buffer to 980 mL distilled water for obtaining a final volume of 1000 mL wash buffer). Label as diluted wash buffer.
Note: Diluted wash buffer must be stored at 2- 8°C (35-46°F).

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 have reached room temperature and form a homogeneous, crystal-free solution before usage. (Alternatively, the Acylation Reagent can be stored at room temperature (20-25°C) separate from the other kit components.
See Storage Information)

Sample preparation

Fish meal:

1. Weigh out **10 g** of fish meal and mix with **240 mL of distilled water**.
2. Stir the suspension for **10 minutes** at room temperature.
3. Pipette 1 mL of the suspension into a centrifuge tube and centrifuge for 5 minutes at 3000 g at room temperature.
4. Use **50 µL** of the supernatant for acylation.

Fresh fish and other fish samples:

1. Weigh out **10 g** of fresh fish and add it to **240 mL of distilled water.**
2. Homogenize the sample mixture for 1 – 2 minutes in a blender.
3. Pipette 1 mL of the suspension into a centrifuge tube and centrifuge for 5 minutes at 3000 g at room temperature.
4. Use **50 µL** of the supernatant for acylation.

Qualitative Determination:

For the qualitative determination, select the control you need from the controls provided with the kit. The kit controls have the following concentrations and are used as cut-off controls: **3, 10, 20, 30, 50, 100 or 300 ppm**

Quantitative Determination:

For the quantitative determination, use the following controls provided with the kit:
Control: 0, 3, 10, 30, 100 and 300 ppm

Acylation

1. Pipette **50 µL** of **Controls** and **Extracts** into the respective wells of the **Master Block.**
2. Add 1.5 mL of **Acylation Buffer** (in 1 pipetting step) to all wells.
3. Pipette 50 µL of **Acylation Reagent** into all wells. (Color changes from yellow to pink) Continue without any delay to step 4.
4. Incubate for **5 minutes** at room temperature (20-25°C) on a shaker with a rotary speed of approx. 600 rpm. Make sure that mixing is complete (slight pink colour). Use 50 µL for the ELISA assay.

Sample specifications

Accuracy (Recovery percentage, %):

Tuna in sunflower oil:	91 – 98%
Tuna in soya oil:	90 – 98%
Anchovy in oil:	101 – 117%

Cross-reactivity:

Histamine:	100%
3-methylhistamine:	0.44%
Tyramine:	0.69%
Cadaverine	0.40%
L-phenylalanine:	<0.02%
L-histidine:	<0.02%
L-tyrosine:	<0.02%
Tryptamine:	<0.02%
L-tryptophan:	<0.02%
Spermine	<0.02%
Putrescine	<0.02%
Trimethylamine	<0.02%

Technical support

Not sure if the test works with your specific samples or matrices? Let our longstanding experience in food allergen testing work for you. Contact our technical sales representative in your region to know more.

ELISA procedure

Before starting

Procedural guidelines:

- Make sure you have everything you need ready before starting the assay.
- All reagents and kit components must be equilibrated to room temperature, i.e. 20-25°C, before use.
- It is good laboratory practice to run standards and sample extracts in duplicates.
- Run a standard curve with each assay.
- Adhere to the incubation times stated in the procedure. Use of incubation times other than those specified may give inaccurate results.
- It is strongly recommended that the assay be performed with an 8-channel pipette.
- The wash procedure is critical and therefore must be performed accurately.
- Do not run more than 2 eight-well strips in one experiment when using an 8-channel pipette.

Precautions:

- Do not mix or interchange reagent lots from different kits lots.
- Due to the high risk of cross-contamination, all used instruments must be cleaned thoroughly before sample preparation. Adhere to the instructions for test procedures.
- Cover or cap all reagents when not in use.
- The stop solution contains acid. Avoid contact with skin or eyes. If exposed, flush with water.
- Wear protective gloves and safety glasses when using the kit.
- Dispose of all materials and containers properly after use.

Assay protocol

1. Pipette 50 µL of **Acylated Controls** and **Acylated Samples** into the wells of the Histamine Microtiter Plate.
2. Pipette **100 µL of Histamine Antiserum Conjugate** into each well with an 8-channel pipette.
3. Incubate for **10 minutes** at room temperature (20-25°C) on a shaker (with a speed of approx. 600 rpm).
4. **Wash step:** Empty the content of the microwell strips into a waste container. Wash by filling each microwell with diluted wash buffer, and then discard it again. Repeat this step 2 times, for a total of **3 washes**.
5. Lay several layers of absorbent paper towels on a flat surface and tap microwell strips on towels to remove all of the residual buffer after the third wash.
   Note: Never insert absorbent paper directly into the wells.
6. Pipette **100 µL of Substrate solution** into each well with an 8-channel pipette.
7. Incubate for **10 minutes** at room temperature (20-25°C) on a shaker (with a speed of approx. 600 rpm). Avoid exposure to direct sun light!
8. Add **100 µL of Stop Solution** to each well with an 8-channel pipette and shake the microtiter plate gently by hand to ensure a homogeneous distribution of the solution.
9. Read the absorbance of each well **within 10 minutes** after the addition of the stop solution at 450 nm with a microplate reader.

   Note: Do not return unused reagents to their original bottles. Carefully note which rows/strips contain controls or samples during the assay.

Results analysis

Qualitative results:

If the absorbance of the sample is higher than that of the Cut-off control, the histamine level in the sample is lower than the Cut-off control.

If the absorbance of the sample is lower than that of the Cut-off control, the histamine level in the sample is higher than the Cut-off control.

Quantitative results:

Results can be easily calculated using the **Romer Labs® spreadsheet** that is provided free of charge upon request. With the Romer Labs® spreadsheet you only need to insert the obtained OD values and the histamine amounts in your samples are calculated automatically.

Alternatively, construct a dose-response curve of the six controls using either the unmodified OD values or the OD values expressed as a percentage of the OD of the zero (0ppm) standard. Since the amount of histamine in each control is known, the unknowns can be measured by interpolation from this standard curve.

Note: If the percent coefficient of variation (%CV) of the duplicate readings of the standards or of the samples exceeds 20%, the result of your test might be inaccurate. Repeat the assay.

Visit **www.romerlabs.com** to find worldwide contact information.
For further information please contact us at:

Romer Labs Division Holding GmbH
Technopark 5
3430 Tulln, Austria
T: +43 2272 61533
office.romerlabseu@dsm-firmenich.com