

Product Manual

AgraQuant® Histamine ELISA test kit **Article number 10002017**

Intended use

The AgraQuant® Histamine ELISA test kit is an immunoassay designed for the quantitative analysis of histamine in food samples. Samples can vary from wine or champagne to milk and cheese, from fish meal to fresh fish and sausage. This product is intended for laboratory use

Performance characteristics

Limit of detection (LOD): 0.15 ppb (0.15 µg/kg) histamine

Limit of quantification (LOQ): 0.1 – 100 ppm (0.1 - 100 mg/kg) histamine (depending on food sample)

Range of quantification: Milk:	0.1 – 10 ppm histamine
Wine, champagne:	0.25 – 25 ppm histamine
Fresh fish, sausage, cheese:	2.5 – 250 ppm histamine
Fish meal:	100 – 10,000 ppm histamine

Plate format: 96 wells

Assay time: sample preparation – 20-35 minutes (approx.)
total incubation time – 55 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 lead to toxic reactions, commonly referred to as “scombroid poisoning”.

Higher histamine levels may also be present in other types of food. People who are intolerant to histamine will suffer from allergy-like reactions from an oral intake of histamine.

Product information

About the ELISA test kit

The AgraQuant® Histamine test kit is a competitive enzyme-linked immunosorbent assay (ELISA) used for the quantification of histamine in food 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 food products. With AgraQuant® Histamine, wine or champagne, milk, cheese and sausage, as well as fresh fish or fish meal can be tested for the presence of histamine.

Storage information

Always store the AgraQuant® Histamine ELISA Test kit 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, 68-77°F) 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 ELISA test kit contains the following items:

- 96 non-coated microwells in a zip-lock bag (reaction plate)
- 96 histamine-coated microwells (12 eight-well strips) in a microwell holder sealed in a foil pouch
- 6 vials of ready-to-use histamine standards (0, 0.5, 1.5, 5, 15, 50 ppb)
- 1 bottles of 22 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 12 mL of histamine-antiserum
- 1 bottle of 12 mL of conjugate solution
- 1 bottle of 12 mL of substrate solution
- 1 bottle of 12 mL of stop solution
- 1 bottle of 4 mL of control 1 (3 ppb ± 1 ppb)
- 1 bottle of 4 mL of control 2 (10 ppb ± 3 ppb)
- 1 piece of plate sealing tape (to be used during the Acylation and ELISA incubation steps)

Materials required but not included

Extraction Procedure:

- Blender or homogenizer
- Analytical balance
- Graduated cylinder, 100 mL
- Distilled or deionized water
- 10 mM PBS buffer
- Containers for sample preparation
- Plate 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
- Microwell reader with 450 and 630 nm filters

ELISA kit – Assay principle

Agra Quant® Histamine ELISA kits are competitive ELISA test kits for the quantitative determination of derivatized histamine in food extracts. 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 limited number of antiserum binding sites. When the system is in equilibrium, free antigen and free antigen-antiserum complexes are removed by the washing step. The conjugate contains an enzyme and binds to the antiserum bound to the solid-phase histamine. 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. 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 and a differential filter between 620 and 650 nm. The measurement must take place within 10 minutes after adding the stop solution.

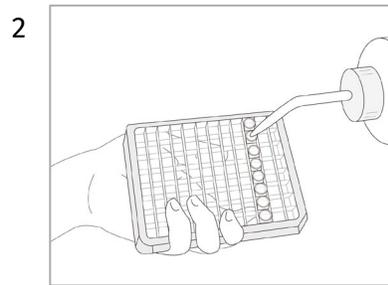
To analyze the results, please refer to *Results analysis* at the end of this product manual.

Protocol at a glance

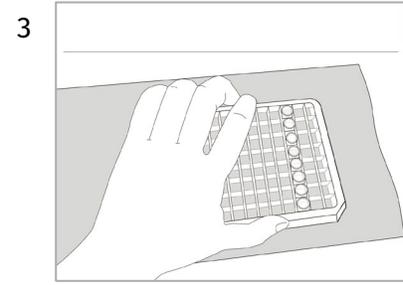
The following section gives only an overview of the ELISA procedure. Before performing the assay, carefully read through this product manual.



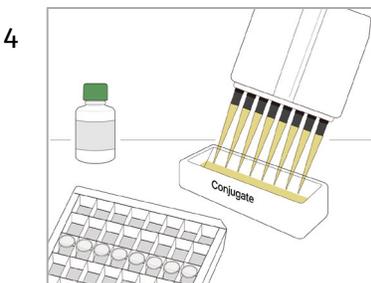
Dispense acylated **samples** and **standards** into the histamine-coated wells. Add histamine antiserum.
Incubate for 30 min.



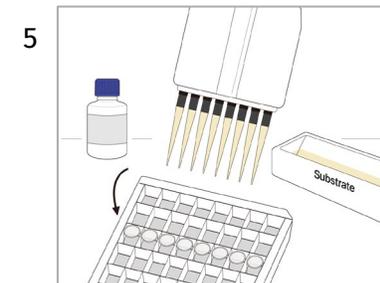
Carefully empty the microwells and wash **3 times** with diluted wash buffer.



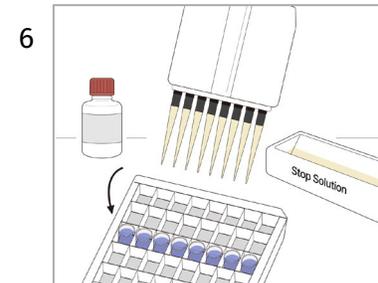
Tap the microwell strips on towels to remove all residual buffer.



Add the **conjugate solution**.
Incubate for 10 min. Wash as in step 2 and 3.



Add the **substrate solution**.
Incubate for 15 min.
 Avoid direct sunlight.



Stop the reaction by pipetting the **stop solution** to each microwell.

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 **1 g** of fish meal and mix with **200 mL of distilled water**.
2. Stir the suspension for **15 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. Pipette **20 µL** of the supernatant into a fresh tube and dilute with **20 mL** of distilled water (Do not use glassware for this dilution step).
5. Use **100 µL** of the solution for acylation.

Fresh fish*, sausage, cheese:

1. Weigh out **10 g** of fresh fish (sausage, cheese) and add it to **90 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. Remove lipid layer by suction! Pipette **20 µL** of the aqueous supernatant into a fresh tube and dilute with **10 mL** of distilled water (Do not use glassware for this dilution step).
5. Use **100 µL** of the solution for acylation.

* For fresh fish we recommend to follow the sample preparation described on **AOAC Official Method 937.07**.

Milk:

1. Pipette **5 mL** milk into a centrifuge tube and centrifuge the sample for 5 minutes at 3000 g at room temperature.
2. Remove lipid layer by suction! Pipette 20 µL of defatted milk into a fresh tube and dilute with 4 mL 10 mM PBS buffer.
3. Use **100 µL** of the solution for acylation

Wine, champagne:

Dilute 20 µL of the sample with 10 mL of distilled water (Do not use glassware for this dilution step) and use **100 µL** of the solution for acylation.

Acylation

1. Pipette **100 µL** of **Standards, Controls** and **Extracts** into the respective wells of the **not coated Reaction Plate**.
2. Add 25 µL of **Acylation Reagent** to all wells.
3. Pipette 200 µL of **Acylation Buffer** into all wells.
4. Incubate for **15 minutes** at room temperature (20-25°C) on a shaker with a rotary speed of approx. 600 rpm.
5. Use **25 µL** for the ELISA assay.

Sample specifications

Accuracy (Recovery percentage, %):

Milk:	83 – 110%
White wine:	94 – 108%
Red wine:	99 – 116%
Tuna:	88 – 97%
Fish meal:	76 – 106%

Cross-reactivity:

Histamine:	100%
3-methylhistamine:	0.01%
Tyramine:	<0.001%
L-phenylalanine:	<0.001%
L-histidine:	<0.001%
L-tyrosine:	<0.001%
Tryptamine:	<0.001%
5-hydroxyindoleacetic acid:	<0.001%
Serotonin:	<0.001%

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 **25 µL of Acylated Standards, Acylated Controls and Acylated Samples** into the wells of the Histamine Microtiter Plate.
2. Pipette **100 µL of Histamine Antiserum** into each well.
3. Incubate for **30 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 Conjugate solution** into each well.
7. Incubate for 10 minutes at room temperature (20-25°C) on a shaker (with a speed of approx. 600 rpm).
8. **Wash step:** Perform the washing step as described above at point 4.
9. 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.
10. Pipette **100 µL of Substrate solution** into each well.

11. Incubate for **15 minutes** at room temperature (20-25°C) on a shaker (with a speed of approx. 600 rpm). Avoid exposure to direct sun light!
12. Add **100 µL of Stop Solution** to each well and shake the microtiter plate gently by hand to ensure a homogeneous distribution of the solution.
13. Read the absorbance of each wells **within 10 minutes** after the addition of the stop solution at 450 nm with a microplate reader, using a reference wavelength between 620 and 650 nm.
Note: Do not return unused reagents to their original bottles. Carefully note which rows/strips contain controls or samples during the assay.

Results analysis

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 standards 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 standard is known, the unknowns can be measured by interpolation from this standard curve.

The histamine concentration in µg/kg (ppb) of each sample is read from the calibration curve and has to be multiplied by the corresponding dilution factor (see below). Control 1 (3 ± 1 ppb) and Control 2 (10 ± 3 ppb) provided in the test kit must be within the acceptance range for the purpose of checking the validity of the assay.

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.

Dilution factors:

- Milk: 200
- Wine, champagne: 500
- Fresh fish, sausage, cheese: 5,000
- Fish meal: 200,000

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