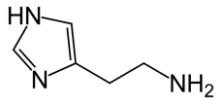
AgraQuant® Histamine Rapid Assay (3 – 300ppm)

Order #: COKAL0548

Intended Use

The AgraQuant® Histamine Rapid Assay is an indirect competitive enzyme-linked immunosorbent assay (ELISA) that determines a quantitative level for the presence of histamine in food.

The AgraQuant® Histamine Rapid Assay is a highly sensitive detection system which is designed for the quantification of histamine in fresh fish, canned fish, salted fish, fish in oil, and fish meal.



Histamine

Histamine

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. Elevated histamine levels (1,000 ppm) can cause gizzard erosion and black vomit in poultry. Histamine testing in fresh fish is a possible control strategy that can be used by seafood processors in their HACCP program. 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 has been associated with many examples of poisoning commonly referred to as "scombroid poisoning," a major health problem for consumers. Scombrotoxic fish usually contains levels of histamine exceeding 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 scombrotoxic fish require the determination of histamine levels in the range of approximately 10 to 200 ppm. Good-quality fish contain less than 10ppm histamine, a level of 30ppm 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[1].

Assay Principles

The assay kit provides materials for the quantitative determination of derivatized histamine in food extracts. The derivatization is part of the preparation of the samples. By use of the acylation reagent, histamine is quantitatively derivatized into N-acyl-histamine. The competitive Histamine ELISA kit uses the microtiter plate format. Histamine is coated to the solid phase of the microtiter plate. 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. An enzyme substrate is added and blue color develops. A stop solution is then added to stop the reaction. The color is then changed from blue to yellow. The microwells are measured optically using a microwell reader with a reading filter of 450 nm. The intensity of the color is inversely proportional to the histamine concentration in the sample.

[1] P. L. Rogers, W. F. Staruszkiewicz, Journal of Aquatic Food Product Technology, Vol. 9 (2) 2000 p. 5 - 17.

Precautions

1. Store reagents at 2-8°C (35-46°F) when not in use, and do not use the kit beyond its expiration date.
2. The Acylation Reagent has a freezing point of 18.5°C. To ensure that the Acylation Reagent is liquid when being used, it must be ensured that the Acylation Reagent has reached room temperature and forms a homogeneous, crystal-free solution before being used. Alternatively, the Acylation Reagent can be stored at room temperature (20 – 25°C) separate from the other kit components.
3. Adhere to incubation times stated in the procedure. Use of incubation times other than those specified may give inaccurate results.
4. Due to high risk of cross contamination, all used instruments must be cleaned thoroughly before sample preparation.
5. The Stop Solution contains acid. Avoid contact with skin or eyes. If exposed, flush with water.
6. Wear protective gloves and safety glasses when using the kit. Dispose of all materials, containers and devices appropriately after use.

Solution preparation

Wash buffer

Dilute the 20 mL Wash Buffer Concentrate with distilled water to a final volume of 1000 mL. Store the diluted Wash Buffer Concentrate (Wash Buffer) at 2 – 8 °C.

Procedure

Sample Preparation / Extraction

Fish meal

1. Weigh 10 g of fish meal in 240 mL of distilled water and stir for 10 minutes at room temperature.
2. Pipette 1 mL of the suspension into an Eppendorf-tube or similar centrifugation tube and centrifuge for 5 minutes at 3,000 x g at room temperature of 20 - 25°C (68 - 77°F).
3. Use 50 µL for the acylation.

Fresh fish

1. Homogenize 10 g of fresh fish in 240 mL of distilled water for 1 – 2 minutes by use of a blender.
2. Pipette 1 mL of the suspension into an Eppendorf-tube or similar centrifugation tube and centrifuge for 5 minutes at 3,000 x g at room temperature of 20 - 25°C (68 - 77°F).
3. Use 50 µL for the 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:

Control: 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

Note: All reagents and kit components must be warmed up to room temperature of 20-25°C (68-77°F) before being used in Acylation and Assay. To avoid contamination, the Master Block is for single use only.

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. (Colour changes from yellow to pink) Continue without any delay to step 4.

4. Incubate 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). Take 50 µL for the ELISA assay.

Assay

It is recommended that an 8-channel pipettor be used to perform the assay. If an 8-channel pipettor is not used (i.e. using only single channel pipettes), it is recommended that no more than a total of 16 samples and controls (2 test strips) be run in one experiment.

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.
3. Incubate 10 min at room temperature (20-25°C) on a shaker (with a speed of approx. 600 rpm).
4. Discard or aspirate the contents of the wells and wash each well 3 times thoroughly with 300 µL **Wash Buffer**. After the last wash, expel residual water by tapping the inverted plate on absorbent paper towel. Dry the bottom of the microwells with a dry cloth or towel.
5. Pipette 100 µL of **Substrate** into each well.
6. Incubate for 10 min at room temperature (20-25°C) on a shaker (with a speed of approx. 600 rpm). Avoid exposure to direct sun light!
7. 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.
8. Read the absorbance of the solution in the wells within 10 minutes, using a microplate reader with a reading wavelength of 450 nm.

Additional Notes: Do not return unused reagents to their original bottles. Do not use reagents from different lot numbers. Carefully keep track of the position of Samples and Controls during the assay.

Interpretation of the results

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

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

Results can also be calculated using the Romer® calculation spreadsheet that is provided (free of charge) upon request.

Performance characteristics

Quantitation range:

Fish meal:	3 – 300 ppm
Fresh fish:	3 – 300 ppm

Accuracy (Recovery percentage, %):

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

Precision (CV%):

Intra-assay variation:	CV% ≤ 9% (n = 10)
Inter-assay variation:	CV% ≤ 7% (n = 10)

Cross Reactivity:

Histamine:	100%
3-methylhistamine:	1.94%
Tyramine:	0.23
L-phenylalanine:	<0.02
L-histidine:	<0.02
L-tyrosine:	<0.02
Tryptamine:	<0.02
L-tryptophan:	<0.02

Materials Supplied With Kit

- 1 piece of 48-well non-coated Master Block in a plastic 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, 300 ppm)
- 1 bottle of 20 mL of wash buffer concentrate (50x)
- 1 bottle of 5.5 mL of Histamine antiserum conjugate
- 1 bottle of 12 mL of substrate solution
- 1 bottle of 12 mL of stop solution
- 2 bottles of 50 mL of acylation buffer
- 1 bottle of 3 mL of acylation reagent. Store at room temperature

Equipment and Materials Required But Not Provided With Kit

- Microtiter plate washing device
- Shaker (shaking amplitude 3mm; approx. 600 rpm)
- Distilled water
- Blender
- *EQOLE1010: Balance, 400 g
- *EQOLE1050: Graduated cylinder: 250 mL
- Centrifuge, or Micro-centrifuge
- *8-channel pipettor capable of pipetting 25 – 200 µL with tips
- *Single channel pipettors capable of pipetting 10 – 100 µL and 100 – 1000 µL with tips
- *EQOLE1300: Timer
- *COKAD1150: Wash bottle
- Absorbent paper towels
- *Reagent boats for use as reagent containers for an 8-channel pipettor
- *Microwell reader with a 450nm filter, (-eg. Stat Fax® 303 Plus manufactured by Awareness Technology Inc.) or equivalent.

*Items available from Romer Labs, Inc.® - Americas Division

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