Megazyme

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HISTAMINE
ASSAY PROCEDURE

K-HISTA 01/20

(100 Assays per Kit)

INTRODUCTION:

High levels of histamine may develop in a variety of foods as they decompose, this is an issue that particularly affects fish. This histamine accumulation is due to the colonisation of certain bacterial species on foods containing high levels of histidine. These bacteria express histidine decarboxylase which converts histidine to histamine. Fish species such as tuna, marlin, sardines, anchovy, herring and mackerel are particualrly prone to histamine accumulation on decomposition due to their high histidine content. Exposure to excess quantities of histamine through the ingestion of spoiled food causes allergy-like food poisoning responses, also known as scombroid poisoning. Scombroid poisoning may show a variety of symptoms, including rash, nausea, vomiting, diarrhoea, constriction of the air passage and muscle weakness. Cases of paralysis and death have also been reported.

As a result of the human illness potential of histamine, strict guidelines are enforced by food regulation authorities to control the levels in foods prone to histamine accumulation. These include rigid storage and processing requirements, documentation of refrigeration records and histamine testing among the Hazard Analysis Critical Control Point (HACCP) programs. The Megazyme Histamine assay kit (**K-HISTA**) is a simple and user friendly method to aid with this compliance.

PRINCIPLE:

The histamine quantification assay is based on the histamine dehydrogenase (HDH) mediated oxidative deamination of histamine. In the presence of the electron carrier 1-Methoxy-5-methylphenazinium methylsulfate (m-PMS) the tetrazolium salt iodonitrotetrazolium chloride (INT) is converted through a series of redox reactions to a coloured formazan product that absorbs at 492 nm. Histamine in the sample is quantified using a single-point standard of known histamine concentration (see page 6).

$$\text{Histamine} + H_2O + HDH_{oxidized} \longrightarrow \text{imidazole acetaldehyde} + NH_3 + HDH_{reduced}$$

$$HDH_{reduced} + \text{m-PMS}_{oxidized} \longrightarrow HDH_{oxidized} + \text{m-PMS}_{reduced}$$

$$\text{m-PMS}_{reduced} + \text{INT} \longrightarrow \text{m-PMS}_{reduced} + \text{formazan (coloured)}$$

SENSITIVITY, LINEARITY AND PRECISION:

The smallest differentiating absorbance for the assay (Limit of Detection) is 0.009 absorbance units, this corresponds to ~ 0.09 μg of histamine per test or ~ 0.75 mg/Kg (ppm) of histamine in a fish sample treated as per

the standard sample preparation example, and using a volume of 1.0 mL in the test. The Limit of Quantification is ~ 0.5 μg of histamine per test or ~ 4.2 mg/Kg (ppm) of histamine in a fish sample, which is derived from an absorbance difference of 0.03 and a sample volume of 1.0 mL in the assay. The assay is linear over the range of 0.5 to 12 μg of histamine per assay, this is shown in Figure 2 (page 8).

INTERFERENCE:

The If the conversion of histamine has been completed within the time specified in the assay (approx. 20 min), it can be generally concluded that no interference has occurred.

Interfering substances in the sample being analysed can be identified by including an internal standard. Quantitative recovery of this standard would be expected. Losses in sample handling and extraction are identified by performing recovery experiments, i.e. by adding histamine to the sample in the initial extraction steps.

Other biogenic amines such as tyramine, putrescine and cadaverine are often present in significant amounts in spoiled foods. The HDH enzyme supplied in this kit exhibits the highest reported substrate specificity for histamine, with no activity on tyramine, putrescine and cadaverine and very low activity on agmatine and 1,3 diaminopropane.[1]

SAFETY:

The general safety measures that apply to all chemical substances should be adhered to.

For more information regarding the safe usage and handling of this product please refer to the associated SDS that is available from the Megazyme website.

KITS:

Kits suitable for performing 100 assays in manual format are available from Megazyme. The kits contain the full assay method plus:

Bottle 1: Buffer (20 mL, pH 9.0) plus sodium azide (0.02% w/v) as a preservative.
Stable for > 2 years at 4°C.

Bottle 2: (x2) INT/m-PMS.
Lyophilised powder.
Stable for > 5 years below -10°C.

Bottle 3: HDH solution (5 mL) in 50% glycerol plus sodium azide (0.02% w/v) as a preservative.
Stable for > 2 years below -10°C.

Bottle 4: Histamine standard solution.
(10 mL, 300 μg/mL) plus sodium azide (0.02% w/v) as a preservative.
Stable for > 2 years; store sealed at 4°C.

PREPARATION OF REAGENT SOLUTIONS/SUSPENSIONS:

1. Use the contents of bottle 1 as supplied.
   Stable for > 2 years at 4°C.
2. Dissolve the contents of one of bottle 2 in 5 mL of distilled water. Shake well and ensure the substrate is thoroughly resuspended. Use immediately or divide into suitable aliquots and store in polypropylene tubes below -10°C.
   Stable for > 2 years below -10°C. Do not dissolve the contents of the second bottle until required.
3. Use the contents of bottle 3 as supplied. Store the bottle in an upright position below -10°C.
   Stable for > 2 years at -10°C.
4. Dilute the histamine standard before use. Dispense 0.1 mL of bottle 4 into a 13 mL polypropylene tube and add 4.9 mL of Sample Extraction Buffer (100 mM EDTA pH 8.0).
   Make fresh on day of use.

NOTE: The histamine standard solution should be tested with every set of assays as it is used in the calculation for histamine concentration in the sample analysed.

PREPARATION OF BUFFER (not supplied):

Sample Extraction Buffer (100 mM EDTA pH 8.0)

Dissolve 37.2 g of EDTA disodium salt (EDTA·2Na·2H_2O ; MW 372.24 g/mol; Sigma-Aldrich cat. no. E5134) in 750 mL of distilled water. Adjust the pH to 8.0 with sodium hydroxide solution. Adjust the volume to 1 L with distilled water. Stable for > 1 month at room temperature.

EQUIPMENT (RECOMMENDED):

1. Polypropylene tubes (30 mL capacity).
2. Disposable plastic cuvettes (1 cm light path, 1.5 mL).
3. Micro-pipettors, e.g. Gilson Pipetman® (100 µL and 200 µL).
4. Positive displacement pipettor, e.g. Eppendorf Multipette®
   - with 5.0 mL Combitip® [to dispense 0.15 mL aliquots of buffer (bottle 1) and 0.1 mL aliquots of INT/m-PMS solution].
   - with 25 mL Combitip® [to dispense 5 mL aliquots of distilled water used for dissolving INT/PMS powder].
5. Volumetric flask (25 mL capacity).
6. Analytical balance.
7. Spectrophotometer set at 492 nm.
8. Vortex mixer (e.g. IKA® Yellowline Test Tube Shaker TTS2).
9. Stop clock.

SAMPLE PREPARATION:

1. Weigh 10 g of fish tissue and homogenise using a sharp knife or fine blender. Weigh precisely 2 g of this tissue into a heat resistant tube (30 mL capacity) with a cap.

2. Add approx. 15 mL of sample extraction buffer and suspend sample well by shaking vigorously.

3. Boil tubes for 20 min with cap fitted tightly.

4. Allow to cool and quantitatively transfer the entire contents into a 25 mL volumetric flask. Bring the volume to precisely 25 mL with sample extraction buffer.

5. Remove turbidity by filtering through 5C filter paper or centrifuging at 10,000 x *g* for 5 min. Use this filtered sample in the assay as described.

A. MANUAL ASSAY PROCEDURE:

Wavelength: 492 nm
Cuvette: 1 cm light path (glass or plastic)
Temperature: 37°C
Final volume: 1.5 mL
Sample solution: 0.5-12 µg of histamine per cuvette (in 1.0 mL sample volume)

Read against air (without a cuvette in the light path) or against water

Pipette into cuvettes	Blank	Sample or Standard
distilled water	0.20 mL	0.20 mL
sample	-	1.0 mL
sample extraction buffer (EDTA)	1.0 mL	-
solution 1 (buffer)	0.15 mL	0.15 mL
solution 2 (INT/mPMS)	0.10 mL	0.10 mL
Mix*, read the absorbances of the solutions (A_1) after 5 min and start the reaction by addition of:		
solution 3 (HDH)	0.05 mL	0.05 mL
Mix* and read the absorbance of the solutions (A_2) at the end of the reaction (approx. 20 min). Solution 2 is light sensitive and should be protected from light if the cuvettes are not stored in a closed system.		

* for example with a plastic spatula or by gentle inversion after sealing the cuvette with a cuvette cap or Parafilm®.

CALCULATION:

A. HISTAMINE CALIBRATION STANDARD:

1. Determine the absorbance difference (A_2-A_1) for both blank and standard. Subtract the absorbance difference of the blank from the absorbance difference of the standard, thereby obtaining $\Delta A_{standard}$ (for an example see below).

2. Calculate M as follows

$$\mathbf{M} = \frac{\text{Histamine (µg)}}{\Delta A_{standard}} \qquad [\mu g/\Delta A_{standard}]$$

Use "M" to calculate the histamine content of the test samples in section B.

Example:

Following the procedure exactly as outlined on page 3 (Preparation of Reagent Solutions, Step 4) where the $\Delta A_{standard}$ measured is 0.34, the "M" value can be calculated as follows:

$$\mathbf{M} = \frac{6\ \mu g}{0.34} = 17.6$$

Note that $\Delta A_{standard}$ may vary.

A. HISTAMINE CONTENT IN SAMPLE:

Determine the absorbance difference (A_2-A_1) for both blank and sample. Subtract the absorbance difference of the blank from the absorbance difference of the sample, thereby obtaining $\Delta A_{histamine}$.

In order to achieve sufficiently accurate results the value of $\Delta A_{histamine}$ should as a rule be at least 0.1 absorbance units, and not more than 0.8 absorbance units.

The concentration of histamine can be calculated as follows:

$$c = \frac{M \times 25 \times 1.0}{1.5 \times 2.0} \times F \times \Delta A_{histamine} \quad [mg/Kg \text{ or } ppm]$$

where:

M = value of histamine standard [$\mu g/\Delta A_{standard}$]
25 = original sample extract volume [mL]
1.0 = Sample volume in assay [mL]
1.5 = Total assay volume [mL]
2.0 = weight of original sample material [g]
F = dilution factor
ΔA = absorbance change of sample

NOTE: These calculations can be simplified by using the Megazyme ***Mega-Calc***™, downloadable from where the product appears in the Megazyme web site (www.megazyme.com).

ADDITIONAL SAMPLE PREPARATION:

1. Sample dilution.

The amount of histamine present in the cuvette (i.e. in the 1.0 mL of sample being analysed) should range between 0.5 and 12 µg. The sample solution must therefore be diluted sufficiently after extraction to yield a histamine concentration between 0.5 µg/mL and 12 µg/mL. Dilutions should be made in sample extraction buffer.

Dilution Table

Estimated concentration of histamine [µg/mL]	Dilution with sample extraction buffer	Dilution factor (F)
< 12	No dilution required	1
12-120	1 + 9	10
120-1200	1 + 99	100

If the value of $\Delta A_{histamine}$ is too low (e.g. < 0.03), results can be reported as 'below LOQ' or < 4.2 ppm.

APPENDIX:

A. Assay Principle - Histamine detection

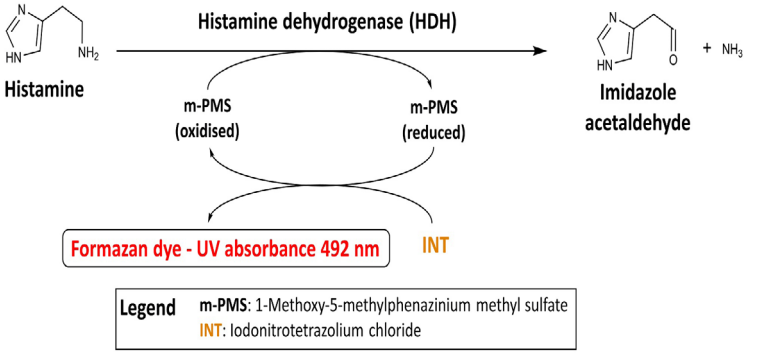

Figure 1. Theoretical basis of the histamine detection assay.

B. Linear range of assay

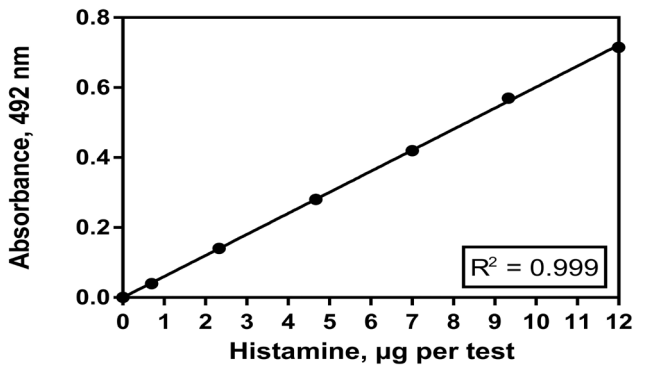

Figure 2. Linearity of the histamine assay.

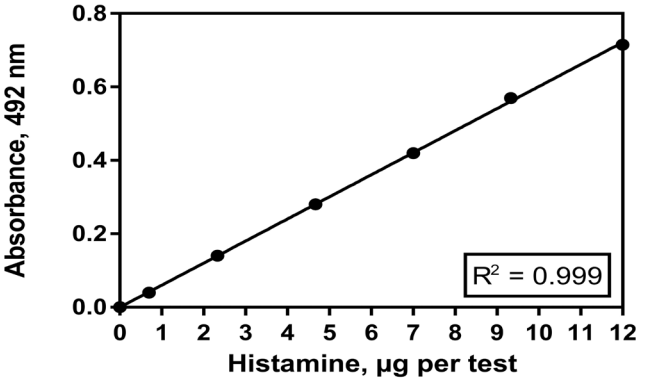

Figure 3. Increase in absorbance at 492 nm on incubation of 0-12 µg of histamine per test.

Sample	Expected Histamine in Spike (mg/L)	Measured Histamine in Spike (mg/L)	Measured Histamine in Sample (mg/L)	Measured Histamine in Sample + Spike (mg/L)	Recovery (%)
Fresh Mackerel	3.7	3.70	0.29	4.00	100.3
Fresh Sardines	3.7	3.73	0.24	3.95	99.5
Tuna in oil	4.2	4.23	1.15	5.45	101.8
Tuna in water	4.2	4.23	1.22	5.39	98.7

Table 1. Percentage recovery of histamine and histamine spike (mg/L) from various fish samples.

	n	µg histamine/ test	Mean, ΔA	Standard Deviation	% CV
Histamine	12	1	0.056	0.0006	1.47
	12	4	0.236	0.0021	1.51
	12	6	0.359	0.0072	2.58
	12	8	0.484	0.0076	1.79
	12	10	0.615	0.0121	2.11
	12	12	0.716	0.0157	2.27

Table 2. Intermediate precision values obtained using a range of histamine standards in the test.

REFERENCES:

1. Bakke, M., Sato, T., Ichikawa, K. & Nishimura, I. (2005). Histamine dehydrogenase from *Rhizobium sp.*: Gene cloning, expression in *Escherichia coli*, characterization and application to histamine determination. *Journal of Biotechnology*, **119**, 260-271.

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