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Product Information

Histamine Quantification Assay Kit

Catalog Number **MAK432**
Storage Temperature -20 °C

TECHNICAL BULLETIN

Product Description

Histamine is a biogenic amine, generated from the single decarboxylation of the amino acid histidine.[1] Histamine food poisonings are allergy-like food poisonings caused by the ingestion of spoiled food containing markedly elevated histamine levels.[2] Elevated levels of bacterial fermentation can lead to elevated histamine in various food and drink sources such as fish, fermented meat, milk, cheese, beer, and wine.[2]

The Histamine Quantification Assay Kit provides a simple and quick colorimetric method for the detection of total histamine from various food sources. The kit is based on the enzymatic oxidation of histamine, which is coupled to the reduction of the formazan WST reagent. The intensity of the product color, measured at 450 nm, is directly proportional to histamine concentration in the sample. The reaction is summarized below:

$$\text{WST-1} + \text{Histamine} \xrightarrow[\text{enzyme}]{\text{Oxidation}} \text{Formazan} + \text{color (450 nm)}$$

The kit has a linear range of 0 to 50 µg/mL (ppm).

Components

The kit is sufficient for 100 colorimetric assays in 96-well plates.

Assay Buffer Catalog Number MAK432A	50 mL
Histamine Standard Catalog Number MAK432B	30 µL
Reaction Enzyme Catalog Number MAK432C	200 µL
Detection Solution Catalog Number MAK432D	200 µL

Reagents and Equipment Required but Not Provided.

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Multiwell plate reader (equipped with a 450 nm filter)
- Flat-bottom 96-well plates
- Methanol (Catalog Number 439193, or equivalent

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Upon receipt, store all components at –20 °C. The unopened kit is stable for 2 years as supplied.

Assay Buffer
Ready-to-use. Upon thawing, store at 2–8 °C.

Histamine Standard
Contains a 5 mg/mL histamine solution. Store at –20 °C.

Reaction Enzyme
Ready-to-use. Avoid freeze/thaw cycles. It is recommended to prepare aliquots, and store the aliquots at –20 °C.

Detection Solution
Ready-to-use. Avoid freeze/thaw cycles. It is recommended to prepare aliquots, and store the aliquots at –20 °C, protected from light.

Preparation Instructions.

Sample Preparation

Note: Histamine may adhere to glass, therefore glassware should be avoided when extracting histamine.

Liquid samples: May be assayed directly.

Solid samples:

1. Prepare Histamine Extraction Buffer: dilute Assay Buffer 1:1(v/v) with 100% methanol.
2. Fish and meat samples (~200-400 mg), should be homogenized with 500 µL Histamine Extraction Buffer.
3. Extraction Samples should then be boiled for 20 minutes at 90 °C, cooled on ice and centrifuged at 10,000 × *g* for 5 minutes.
4. Collect the supernatant.

Procedure

Notes:

- The assay is formatted for a 96-well microplate.
- All standards and samples should be run in duplicate.
- Equilibrate all reagents to room temperature before use.
- A fresh set of standards should be prepared for every use.
- Briefly centrifuge vials before opening.
- All assays (samples, standards and blank) require 50 µL of sample for each reaction (well). Therefore, complete the volume to 50 µL if required. When required, samples should be diluted in Assay Buffer.
- For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.
- **For convenience, an Excel-based calculation sheet is available on the MAK432 Product Detail Page.** Use this sheet to calculate the amounts of reagents required, as well as to calculate the test results.

Standard Curve Preparation

1. Prepare a 50 µg/mL (50 ppm) histamine standard solution by diluting 4 µL of the 5 mg/mL Histamine Standard with 396 µL of Assay Buffer.
2. Prepare a histamine standard curve: To a 96-well plate, add 50 µL of Assay Buffer to each of wells B1-B2 through H1-H2. Add 100 µL of the 50 ppm histamine standard solution to wells A1-A2. Serially dilute histamine by transferring histamine solution as follows: Transfer 50 µL from wells A1-A2 to wells B1-B2. Pipette up and down 10 times, then transfer 50 µL from wells B1-B2 to wells C1-C2. Continue serial dilutions by transferring 50 µL in a similar manner through wells G1-G2. Pipette up and down 10 times, then discard 50 µL from wells G1-G2. Do not transfer to wells H1-H2. This dilution process is summarized in Table 1.

Table 1.
Preparation of Histamine Standards

Wells*	Assay Buffer**	Histamine Standard**	Histamine Concentration (ppm)
A1, A2	-	100 µL of 50 ppm	50.0
B1, B2	50 µL	50 µL from wells A1, A2	25.0
C1, C2	50 µL	50 µL from wells B1, B2	12.5
D1, D2	50 µL	50 µL from wells C1, C2	6.3
E1, E2	50 µL	50 µL from wells D1, D2	3.1
F1, F2	50 µL	50 µL from wells E1, E2	1.6
G1, G2	50 µL	50 µL from wells F1, F2***	0.8
H1, H2	50 µL	-	0.0 (blank)

* Two adjacent wells
** Per well, in duplicate
*** Discard 50 µL from wells G1-2. Do not transfer to wells H1-2.

Reaction Mix
Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µL of Reaction Mix according to Table 2.

Table 2.
Preparation of Reaction Mix

Reagent	Volume
Assay Buffer	46 µL
Reaction Enzyme	2 µL
Detection Solution	2 µL

Assay Procedure
1. Add 50 µL of sample to each sample well.
2. Add 50 µL of the Reaction Mix to each of the standard and sample wells.
3. Incubate the reaction for 30 minutes at room temperature. Measurement up to 60 minutes is possible. Protect the plate from light during the incubation.
4. Measure the absorbance (A) at 450 nm.

Results

Note: An Excel-based calculation sheet is available at the MAK432 Product Detail Page. Use this sheet to calculate the test results.

If the Excel-based calculation sheet at the Product Detail Page is not used, calculations should be performed as follows:

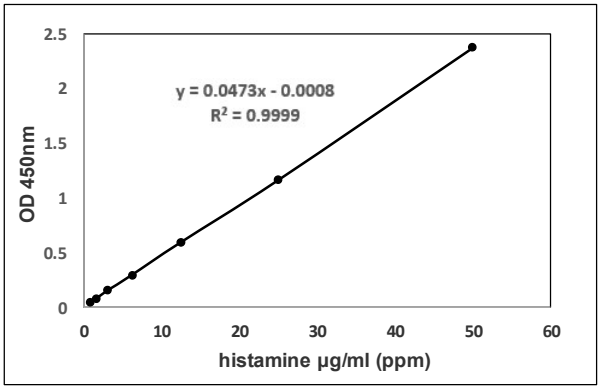
1. Subtract the blank value (no standard; H1,H2) from all standards and sample values.
2. Plot the absorbance measured for each standard against the standard amount per well.
3. Determine the linear regression equation, and use it to calculate the histamine concentration of the sample:

[(Sample)/(Sample volume)] × DF = ppm histamine

Sample = Amount of histamine in unknown sample (ppm), calculated from the standard curve.
Sample volume = Sample volume added into the wells (50 µL)

DF = Sample dilution factor (if sample is not diluted, the DF value is 1)

Figure 1.
Typical histamine standard curve using MAK432 kit and protocol.



References
1. Pino-Ángeles, A., et al., *Biomedical Aspects of Histamine*, 33-57, ed. Shahid, M., et al., Springer Netherlands (2011).
2. Morrow, J.D., et al., Evidence that histamine is the causative toxin of scombroid-fish poisoning, *N. Engl. J. Med.,* **324**, 716–720 (1991).

HM, NA, ER, VC 10/20-1