Technical Manual

ChromaDazzle Histamine Assay Kit

Catalogue Code: BA0120

Pack Size: 100 assays

Research Use Only

DESCRIPTION

HISTAMINE ($C_5H_9N_3$) is a biogenic amine naturally present in many foods and body cells in amounts without toxicological significance. It is also a contaminant that mostly found in the Scombridae family fishes such as tuna and mackerel or other seafood products when improperly handled or stored. The consumption of foods containing high level of histamine may lead to an allergy-like food poisoning known as scombroid poisoning.

Assay Genie ChromaDazzle Histamine Assay Kit is based on histamine dehydrogenase catalyzed oxidation of histamine in which the formed electron mediator reduces a formazan (MTT) reagent. The intensity of product color, measured at 565 nm is directly proportional to histamine concentration in the sample.

KEY FEATURES

Fast and sensitive. Use of 20 µL sample. Linear detection range 0.0025 to 1 mM histamine in 96-well plate assay.

Convenient. The procedure involves adding a single working reagent, and reading the absorbance after 30 minutes. Room temperature assay. No 37°C heater is needed.

High-throughput. "Add-mix-read" type assay. Can be readily automated as a high-throughput 96-well plate assay for thousands of samples per day.

APPLICATIONS

Direct Assays: histamine in food, beverage, and agricultural products.

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Assay Buffer: 10 mL **Standard:** 1.0 mL 20 mM Histamine
HDH Enzyme: 120 µL

Storage conditions. The kit is shipped on ice. Store all components at -20°C upon receiving. Shelf life: 6 months after receipt.

Precautions: reagents are for research use only. Briefly centrifuge tubes before opening. Equilibrate all components to room temperature prior assay. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

Sample Preparation: clear and slightly colored samples can be assayed directly. It is prudent to test several dilutions to determine an optimal dilution factor *n*.

Solid samples (fish, meat, etc) can be homogenized in 0.05% acetic acid (800 µL of 0.05% acetic acid per 200 mg sample is typically sufficient) followed by filtration or centrifugation (e.g. 10 min 14,000 rpm). Cheese samples should be heated at 60˚C in 0.05% acetic acid to melt and then homogenized.

Beverage samples can be assayed directly. Check the pH of the sample and adjust to 6-8 with NaOH or HCl. Samples containing carbon dioxide should be degassed by gentle stirring prior assay.

Procedure using 96-well plate

1. *Standards*. Prepare 200 µL 1 mM Premix by mixing 10 µL of the Standard (20 mM) and 190 µL distilled water. Dilute standards in 1.5-mL centrifuge tubes as described in the Table.

No	Premix + H_2O	Histamine (mM)
1	100 µL + 0 µL	1.0
2	60 µL + 40 µL	0.6
3	30 µL + 70 µL	0.3
4	0 µL + 100 µL	0

2. Transfer 20 µL standards into separate wells of a clear, flat-bottom 96-well plate. Transfer 20 µL of each sample into two separate wells, one serving as a sample blank well (OD_{BLANK}) and one as a sample well (OD_{SAMPLE}).

3. Prepare sufficient Working Reagent (WR) for all sample and standard wells by mixing, for each well: 85 µL Assay Buffer plus 1 µL HDH Enzyme.

   Add 80 µL WR to the *four Standards* and the *Sample Wells.* Add 80 µL Assay Buffer (*i.e. no HDH Enzyme*) to the *Sample Blank Wells*. Tap plate to mix briefly and thoroughly. Incubate 30 minutes at room temperature.

3. Read optical density at 565 nm (520-600 nm).

CALCULATION

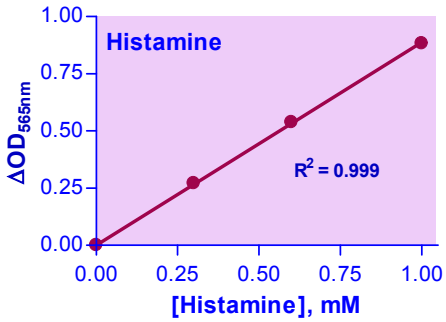
Subtract the blank value (#4) from the standard values and plot the ΔOD against standard concentrations. Determine the slope and calculate the histamine concentration of Sample,

$$[\text{Histamine}] = \frac{OD_{SAMPLE} - OD_{BLANK}}{\text{Slope (mM}^{-1})} \times n \quad (\text{mM})$$

OD_{SAMPLE} and OD_{BLANK} are optical density readings of the Sample and Sample Blank, respectively. *n* is the sample dilution factor.

Note: if the sample OD value is higher than OD for 1 mM histamine standard, dilute sample in water and repeat the assay. Multiply the results by the dilution factor.

Conversions: 1 mM histamine equals 11.1 mg/dL, or 111 ppm.



Standard Curve in 96-well plate assay in water

EXAMPLES. The results below are obtained on particular samples, and may differ from sample to sample.

Sample	Measured Histamine (ppm)
Putrid Mackerel	7783 ± 115
Fresh Tuna	24.0 ± 0.6
Day Old Tuna	209 ± 1
Fish Sauce	99.4 ± 0.5
Red Wine	3.5 ± 0.1
Parmesan Cheese	101.8 ± 3.8
Avocado	3.8 ± 0.2

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices, centrifuge tubes, clear flat-bottom 96-well plates (e.g. VWR cat# 82050-760), and plate or cuvette reader.

LITERATURE

1. Zarei, Mehdi, et al (2010). Histamine and Heavy Metals Content of Canned Tuna Fish. Global Veterinaria. 5 (5): 259-263.
2. Taylor, Steve L., and Ronald R. Eitenmiller (1986). Histamine food poisoning: toxicology and clinical aspects. CRC Critical Reviews in Toxicology 17.2: 91-128.
3. ZaMaN, Muhammad Zukhrufuz, et al (2010). Occurrence of biogenic amines and amines degrading bacteria in fish sauce. Czech Journal of Food Science 28.5: 440-449.

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