

Histamine Assay Kit (Colorimetric)

LS-K175-100 (100 Tests) • Store at -20°C



Introduction

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.

LSBio's 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 μ L 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.

Components

Component	K175-100
	100 Tests
Assay Buffer	10 mL
HDH Enzyme	120 μ L
Standard (20 mM Histamine)	1.0 mL

Materials Not Supplied

Pipetting devices, centrifuge tubes, clear flat-bottom 96-well plates and plate or cuvette reader.

Storage

The kit is shipped on ice. Store all kit components at -20 °C.

FOR RESEARCH USE ONLY! Not for use in humans.

LifeSpan BioSciences, Inc. • 2401 Fourth Avenue, Suite 900, Seattle, WA 98121

www.LSBio.com • (206) 464-1554 • TechnicalSupport@LSBio.com

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Assay Procedure

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 μ L 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

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

No	Premix + H ₂ O	Histamine (mM)
1	100 μ L + 0 μ L	1.0
2	60 μ L + 40 μ L	0.6
3	30 μ L + 70 μ L	0.3
4	0 μ L + 100 μ L	0

- Transfer 20 μ L standards into separate wells of a clear, flat-bottom 96- well plate. Transfer 20 μ L of each sample into two separate wells, one serving as a sample blank well (OD_{BLANK}) and one as a sample well (OD_{SAMPLE}).
- Prepare sufficient Working Reagent (WR) for all sample and standard wells by mixing, for each well: 85 μ L Assay Buffer plus 1 μ L HDH Enzyme. Add 80 μ L WR to the four Standards and the Sample Wells.
Add 80 μ L 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.
- Read optical density at 565 nm (520-600 nm).

Calculations

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

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

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.

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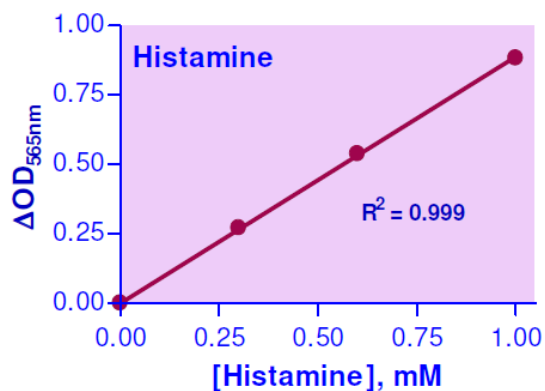
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Sample Data



Standard Curve in 96-well plate assay in water

Version: V.08.09.2018

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