CREATIVE BIOMART®
Assay Kit

Histamine Assay Kit (Colorimetric)

Product Information

Cat.No. Kit-1005

Product Overview

Histamine Assay Kit can thus be used to identify dangerous levels of histamine in various fish and meat products, as well as other fermented food and sauces/beverages. With our kit, histamine can be detected in samples at concentrations as low as 10 µM, or 10 µg/ml (10 ppm).

Description

Histamine is a biogenic amine with considerable biological relevance. As a signaling molecule histamine plays manifold roles in the body, ranging from local immune responses to neurotransmission. Histamine is also frequently encountered in food, particularly fish and fermented food products such as sauerkraut and aged cheeses. This is because some bacteria generate histamine from histidine via the enzyme histidine decarboxylase. Elevated levels of bacterial fermentation can lead to elevated histamine in raw meat and food products. Histamine levels thus can be used as an indicator of spoilage. While the body is accustomed to exposure to low levels (30-50 ppm, or mg/l) of histamine, ingestion of food containing levels higher than several hundred parts per million can lead to adverse effects. This is known as histamine poisoning.

Applications

Estimation histamine content of various food products.

Storage

Store kit at -20°C, protected from light. Briefly spin small vials prior to opening. Read entire protocol before performing the assay.
Histamine Assay Buffer: Store at -20°C or 4°C. Bring to room temperature (RT) before use.
Histamine Probe: Bring to room temperature and resuspend in 220 µl Histamine Assay Buffer. Store at -20°C. Bring to RT before use.
Histamine Enzyme Mix: Reconstitute with 220 µl Histamine Assay Buffer. Aliquot and store at -20°C. Use within 2 months.
Histamine Standard: Bring to room temperature before use. Store at -20°C.

Tel: 1-631-559-9269 1-516-512-3133
Email:info@creative-biomart.org
Fax:1-631-938-8127
45-1 Ramsey Road, Shirley, NY 11967, USA

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Size

100 assays

Kit Components

Histamine Assay Buffer 25 ml
Histamine Enzyme Mix 1 vial
Histamine Probe 1 vial
Histamine Standard (50 mM) 100 µl

Materials Required but Not Supplied

96-well clear plate with flat bottom
10 kDa Spin Column
Multi-well spectrophotometer
100% Methanol

Compatible Sample Types

Fish Sauce
Canned or Frozen Fish or other Meat products
Milk or Yogurt
Cheese

Assay Protocol

1. Sample Preparation:
Prepare Histamine Sample Buffer by diluting Histamine Assay Buffer 1:1 with 100% Methanol. Liquid samples may be assayed directly; see notes below. For fish and meat samples, homogenize (~200-400 mg) samples using 500 µl Histamine Sample Buffer. Boil sample for 20 minutes at 90°C in sealed tubes, then cool on ice. Centrifuge samples at 10,000 X g for 5 min. Collect the supernatant. Dilute samples, if necessary, using Histamine Assay Buffer & add 5-10 µl into desired well(s) in a 96-well plate. Adjust the volume to 50 µl/well with Histamine Assay Buffer.
Notes:
a. Histamine concentration varies over a wide range depending on the sample. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings

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are within the Standard Curve range.
b. Components of some samples may interfere with the assay. To reduce background, it is recommended to dilute samples with Histamine Assay Buffer. If interference is observed in the diluted samples, prepare parallel sample well(s) as sample background control(s) and make up the volume to 50 µl/well with Histamine Assay Buffer.
c. For samples having high protein content, we recommend deproteinizing the samples (tissue or cell lysate or biological fluids) using 10 kDa Spin Column (Cat. # 1997 or equivalent). Add sample to the spin column, centrifuge at 10,000 X g, 4°C for 10 min. Collect the filtrate.
d. To ensure accurate determination of Histamine in the test samples or for samples having low concentrations of Histamine, we recommend spiking samples with a known amount of Histamine Standard (e.g. 4 nmol) and running them in parallel with unspiked samples.
2. Standard Curve Preparation:
Prepare 1 mM Histamine Standard by adding 10 µl of 50 mM Histamine Standard to 490 µl of ddH2O. Add 0, 1, 2, 4, 6, and 8 µl of 1 mM Histamine Standard into a series of wells in a 96-well plate to generate 0, 1, 2, 4, 6 and 8 nmol of Histamine/well. Adjust the volume to 50 µl/well with Histamine Assay Buffer.
3. Reaction Mix:
Mix enough reagents for the total number of wells to be assayed. For each well, prepare 50 µl of Reaction Mix containing:

Reaction Mix	*Background Control Mix
Histamine Assay Buffer	46 µl	48 µl
Histamine Enzyme Mix	2 µl	----
Histamine Probe	2 µl	2 µl

Mix well. Add 50 µl of Reaction Mix into Standard and sample wells. Mix.
* For samples having background, add Background Control Mix to background control well(s) and mix.
4. Measurement:
Incubate plate at 37°C for 30 min. Measure absorbance at 450 nm in end point mode.
5. Calculation:
Subtract 0 Histamine Standard reading from all readings. Plot the Histamine Standard Curve. If

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sample background control is significant, then subtract sample background control reading from sample reading to obtain corrected absorbance. Apply corrected absorbance to Standard Curve to get B nmol Histamine in the sample well.

Sample Histamine Concentration (C) = B/V X D nmol/µl or mM

Where: B is amount of Histamine in the sample well from Standard Curve (nmol)

V is sample volume added into the reaction well (µl)

D is sample dilution factor

Note: For spiked samples, correct for any sample interference by using the following equation: Histamine amount in spike sample well (B)= ODsample (corrected) / [(ODsample+Histamine Std (corrected))−(ODsample (corrected))] □ Histamine spike (nmol) Histamine molecular weight: 111.15 g/mol

1 mM ≡ 111.15 ppm

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