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BioVision
BioVision Incorporated

Histamine Assay Kit (Fluorometric)

(Catalog # K386-100; 100 assays; Store at -20°C)

I. Introduction:

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. Concentrations of histamine in the human blood are typically in the low nM range and only in instances of acute histamine poisoning would this metabolite be elevated above the sub-micromolar range. BioVision's Histamine Assay Kit can be used to identify low levels of histamine in various fish and products and sauces/beverages, as well as some biological samples. With our kit, as little as 10 pmoles of histamine can be detected in samples, or roughly 10 parts per billion (ppb).

Histamine —(Enzyme Mix)→ Intermediate —(Developer/Probe)→ Fluorescence (Ex/Em= 535/587 nm)

II. Application:

- Estimation of histamine content of various food products and biological samples.

III. Sample Type:

- Meat and Food Products
- Soy Sauce and other condiments
- Biological samples (saliva e.g.) and lysates

IV. Kit Contents:

Components	K386-100	Cap Code	Part Number
Histamine Assay Buffer	25 ml	WM	K386-100-1
Histamine Enzyme Mix	1 vial	Purple	K386-100-2
Histamine Developer	1 vial	Green	K386-100-3
Histamine Probe	0.2 ml	Red	K386-100-4
Sample Clean-Up Mix	1 vial	Blue	K386-100-5
Histamine Standard (50 mM)	100 µl	Yellow	K386-100-6

V. User Supplied Reagents and Equipment:

- 96-well black plate with flat bottom
- 10 kDa Spin Column (Cat. # 1997 or equivalent)
- Multi-well spectrophotometer
- 100% Methanol
- 1 M Tris Base solution

VI. Storage Conditions and Reagent Preparation:

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. Thaw and bring to room temperature (RT) before use.
- **Histamine Probe:** Ready to use as supplied. Store at -20°C. Bring to room temperature before use, protect from light.
- **Histamine Enzyme Mix and Histamine Developer:** Reconstitute each vial with 220 µl Histamine Assay Buffer. Store at -20°C; thaw before use. Use within 2 months.
- **Histamine Standard:** Thaw and bring to room temperature before use. Store at -20°C.

VII. Histamine Assay Protocol:

1. **Sample Preparation:** Prepare Histamine Sample Buffer by diluting Histamine Assay Buffer 1:1 with 100% Methanol. **For liquid samples** carrying low levels of histamine, e.g. soy sauce: treat with 5% Trichloroacetic acid (TCA) by adding 2.5 volumes to 1 volume sample and then agitating the mixture for 5 min. After agitation, filter through a 0.45 µm sterile filter and neutralize with one quarter volume of 1 M Tris. **For fish and meat samples**: homogenize (~50-100 mg) samples using 100 µl Histamine Sample Buffer. Spin down sample for 5 min. at 10,000 x *g*, proceed with supernatant. Add equal volume Histamine Sample Buffer, vortex, and boil sample for 20 minutes at 90°C in sealed tubes. 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 black 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 are within the Standard Curve range.

   b. Endogenous 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 serum, e.g.) 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. In cases where high background remains, sample may be treated with Sample Clean-Up Mix by adding 2 µl Sample Clean-Up Mix to 100 µl Sample and incubation at 37°C for 30 min. Boil sample at 90°C for ten minutes and filter through a 0.2 µm filter before proceeding with Assay.

e. 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. 100 pmol) 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 ddH_2O. Further dilute the 1 mM Histamine Standard by adding 10 µl to 190 µl Assay Buffer to generate a 50 µM Histamine Standard. Add 0, 2, 4, 6, 8, and 10 µl of 50 µM Histamine Standard into a series of wells in a 96-well plate to generate 0, 100, 200, 300, 400, and 500 pmol (0.5 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	45.6 µl	47.6 µl
Histamine Enzyme Mix	2.0 µl	----
Histamine Developer	2.0 µl	2.0 µl
Histamine Probe	0.4 µl	0.4 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 fluorescence at 535 nm excitation/587 nm emission in end point mode.

5. **Calculation:** Subtract 0 Histamine Standard reading from all readings. Plot the Histamine Standard Curve. If 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 pmol/µl or µM

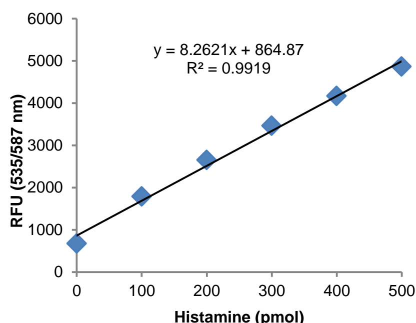
Where: **B** is amount of Histamine in the sample well from Standard Curve (pmol)
V is sample volume added into the reaction well (µl)
D is sample dilution factor

Note: For spiked samples, correct for any sample interference by using the following equation:

$$\textbf{Histamine amount in spike sample well (B)} = \frac{\mathrm{RFU}_{\mathrm{sample\ (corrected)}}}{(\mathrm{RFU}_{\mathrm{sample+Histamine\ Std\ (corrected)}}) - (\mathrm{RFU}_{\mathrm{sample\ (corrected)}})} * \text{Histamine spike (nmol)}$$

Histamine molecular weight: 111.15 g/mol
1 µM ≡ 111.15 ppb

a)



b)

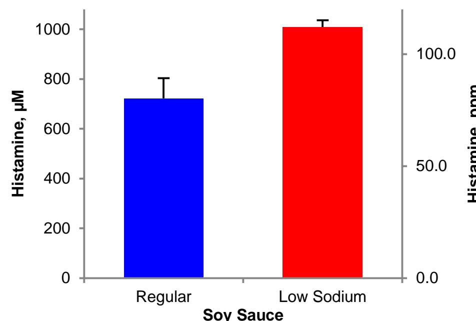


Figure: (a) Histamine Standard Curve. **(b)** Estimation of Histamine concentration in soy sauce. Soy sauce was prepared according to above protocol. Histamine concentrations are: Regular Soy Sauce: 720 ± 100 µM, Low Sodium Soy Sauce: 1010 ± 20 µM.

VIII. Related Products:

Nitric Oxide Colorimetric Assay Kit (K262)
Nitric Oxide Fluorometric Assay Kit (K252)
Histidine Decarboxylase Antibody (3691)
Histamine Colorimetric Assay Kit (K506)
Choline/Acetylcholine Quantification Assay Kit (K625)
α-Ketoglutarate Colorimetric/Fluorometric Assay Kit (K677)
Total Polyamine Assay Kit (Fluorometric) (K475)
Diamine Oxidase Activity Assay Kit (K496)Dounce Tissue Homogenizer (1997)

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