
Product Manual

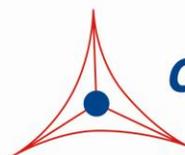
Histamine Assay Kit

Catalog Number

AKR-360

96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures



CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Histamine is a biogenic amine found in the tissues of many different living organisms. In humans, while histamine has been detected in the intestines, brain, lung, and stomach, it is predominately stored in mast cells, neurons, and basophils. Histamine can affect various cellular functions through interaction with G-protein coupled histamine receptors H1, H2, or H3 on target cells. The H1 receptors are largely found in brain cells where they regulate attention, cognition, and circadian rhythm. H1 receptors are also found in peripheral tissues where they control allergy-related bronchial and vascular muscle responses. H2 receptors, expressed throughout the body, are mainly involved in gastric acid secretion, while H3 receptors play a role in neuronal signaling as well as inflammatory process initiation.

While histamine is produced naturally in the body, exposure to higher levels of histamine can occur through the consumption of various food and drink sources such as fish, fermented meat, milk, cheese, beer, and wine. Ingested histamine causes intestinal smooth muscle contraction, local blood vessel dilation, and ultimately symptoms that are similar to an allergic response. High levels of histamine have been correlated with incidence of diseases such as colorectal neoplasms, ulcerative colitis, and Crohn's disease.

Assay Principle

Cell Biolabs' Histamine Assay Kit provides a convenient colorimetric method for the detection of total histamine from extracted food sources. The provided reagents are sufficient for the evaluation of 96 assays. The unknown samples or Histamine standards are added to a 96 well plate followed by the Colorimetric Probe Mix containing WST-1, an electron mediator, and Histamine Dehydrogenase (HDH). During a brief incubation the WST-1 is converted to the formazan form (Figure 1) and the absorbance of the plate is read at 450 nm. The content of Histamine in the unknown samples is determined by comparison with a predetermined Histamine standard curve.

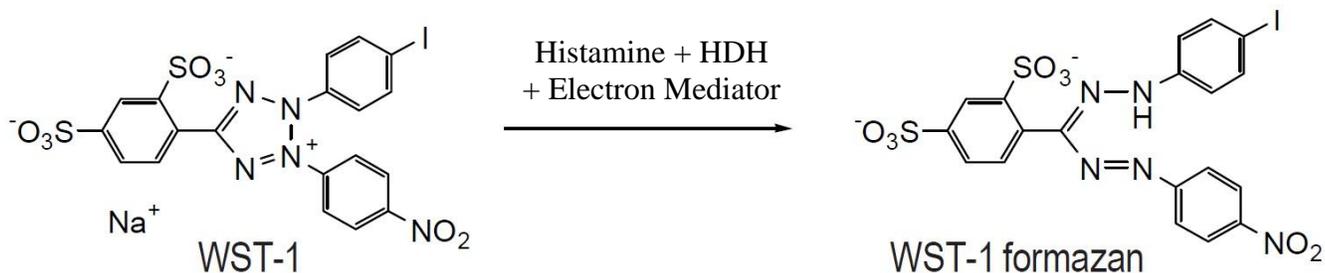


Figure 1. Assay Principle.

Related Products

1. AKR-350: Aflatoxin Competitive ELISA Kit
2. AKR-351: Aflatoxin DNA Adduct Competitive ELISA
3. STA-301: OxiSelect™ BPDE Protein Adduct ELISA Kit
4. STA-357: OxiSelect™ BPDE DNA Adduct ELISA Kit

Kit Components

1. 10X Colorimetric Probe (Part No. 436001): Two 1 mL amber vials
2. Histamine Standard (Part No. 436002): One 20 μ L vial containing 5 mg/mL Histamine
3. Histamine Dehydrogenase (2000X) (Part No. 436004): One 15 μ L vial at 17 U/mL

Note: One unit is defined as the amount of enzyme that produces 1 μ mol of 4-imidazolylacetaldehyde per minute at 37 °C and pH 9.0.

4. 10X Assay Buffer (Part No. 436003): One 35 mL bottle

Materials Not Supplied

1. Food samples containing histamine such as fish, fermented meat, milk, cheese, beer, and wine
2. Methanol
3. 10 μ L to 1000 μ L adjustable single channel micropipettes with disposable tips
4. 50 μ L to 300 μ L adjustable multichannel micropipette with disposable tips
5. Multichannel micropipette reservoir
6. Microplate reader capable of reading at 450 nm (620 nm as optional reference wavelength)

Storage

Upon receipt, store the Histamine Dehydrogenase (2000X) and the Histamine Standard at -80°C. Store the 10X Assay Buffer and the 10X Colorimetric Probe at 4°C.

Preparation of Reagents

- 1X Assay Buffer: Dilute 10X Assay Buffer to 1X with deionized water and store at room temperature.
- Extraction Buffer: Mix 1X Assay Buffer with Methanol at 1:1 (v/v) and store at room temperature.
- Colorimetric Probe Mix: Dilute both the 10X Colorimetric Probe and the Histamine Dehydrogenase (2000X) to 1X concentration in 1X Assay Buffer. For example, for 20 assays add 400 μ L of 10X Colorimetric Probe and 2 μ L of Histamine Dehydrogenase (2000X) to 3.6 mL of 1X Assay Buffer. Store unused Colorimetric Probe Mix at -80°C and thaw at 4°C just prior to use.

Preparation of Standard Curve

Prepare a dilution series of Histamine standards in the concentration range of 0 to 50 μ g/mL (ppm) by diluting the Histamine Standard into Extraction Buffer (Table 1).

Standard Tubes	5 mg/mL Histamine Standard (µL)	Extraction Buffer (µL)	Histamine (µg/mL or ppm)
1	4	396	50
2	200 of Tube #1	200	25
3	200 of Tube #2	200	12.5
4	200 of Tube #3	200	6.25
5	200 of Tube #4	200	3.13
6	200 of Tube #5	200	1.56
7	200 of Tube #6	200	0.78
8	200 of Tube #7	200	0

Table 1. Preparation of Histamine Standards

Preparation of Samples

The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design. Do not use glassware for extraction. Histamine may adhere to glass which could affect the results.

- Meat or seafood: Homogenize 10 to 20 grams of the sample. Transfer 0.5 gram of the homogenate to a centrifuge tube and add 1 mL of methanol. Vortex for 2 minutes. Incubate the homogenate at 75°C for 5 minutes. Vortex for 5 seconds and centrifuge for 5 minutes at 4,000 x g. Transfer 0.25 mL of the supernatant to a new tube, avoiding transfer of any insoluble material. Add 0.25 mL of 1X Assay Buffer to the supernatant and store at 4°C until ready to use.
- Fish meal: Homogenize 10 to 20 grams of the sample. Transfer 0.5 gram of the homogenate to a centrifuge tube and add 2.0 mL of 1X Assay Buffer. Vortex for 2 minutes and centrifuge for 5 minutes at 4,000 x g. Transfer 1.0 mL of the supernatant to a new centrifuge tube, avoiding transfer of any insoluble material. Incubate the supernatant at 75°C for 5 minutes. Vortex for 30 seconds and then centrifuge for 5 minutes at 4,000 x g. Transfer 0.5 mL of the supernatant to a new centrifuge tube, avoiding transfer of any insoluble material. Add 0.5 mL of methanol and incubate the sample at 75°C for 5 minutes. Centrifuge for 5 minutes at 4,000 x g. Transfer 0.5 mL of the supernatant to a new tube and store at 4°C until ready to use.
- Milk: Centrifuge the milk sample for 5 minutes at 4,000 x g. Transfer the lower layer to a clean tube, avoiding contamination from the top layer. Dilute the sample as necessary into Extraction Buffer and store at 4°C until ready to use.
- Wine: Dilute the sample as necessary into Extraction Buffer and store at 4°C until ready to use.

Assay Protocol

1. Prepare and mix all reagents thoroughly before use. Each sample, unknown and standard should be assayed in duplicate.
2. Add 50 μL of unknown sample or Histamine standard to the wells of a 96 well plate.
Note: If needed, unknown samples may be diluted in 1X Assay Buffer.
3. Add 200 μL of the Colorimetric Probe Mix to each well.
4. Incubate 1 hour at 37°C.
5. Read absorbance of each well on a microplate reader using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate typical Histamine Assay Kit results. One should use the data below for reference only. This data should not be used to interpret actual results.

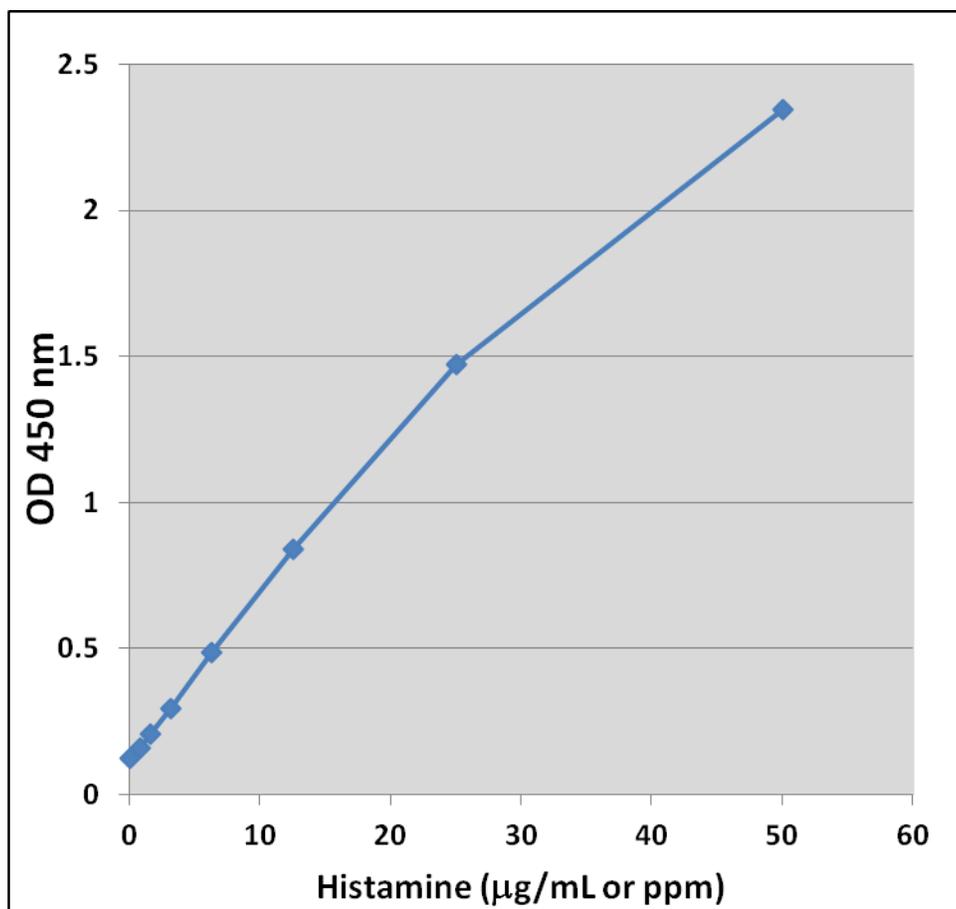


Figure 2: Histamine Standard Curve

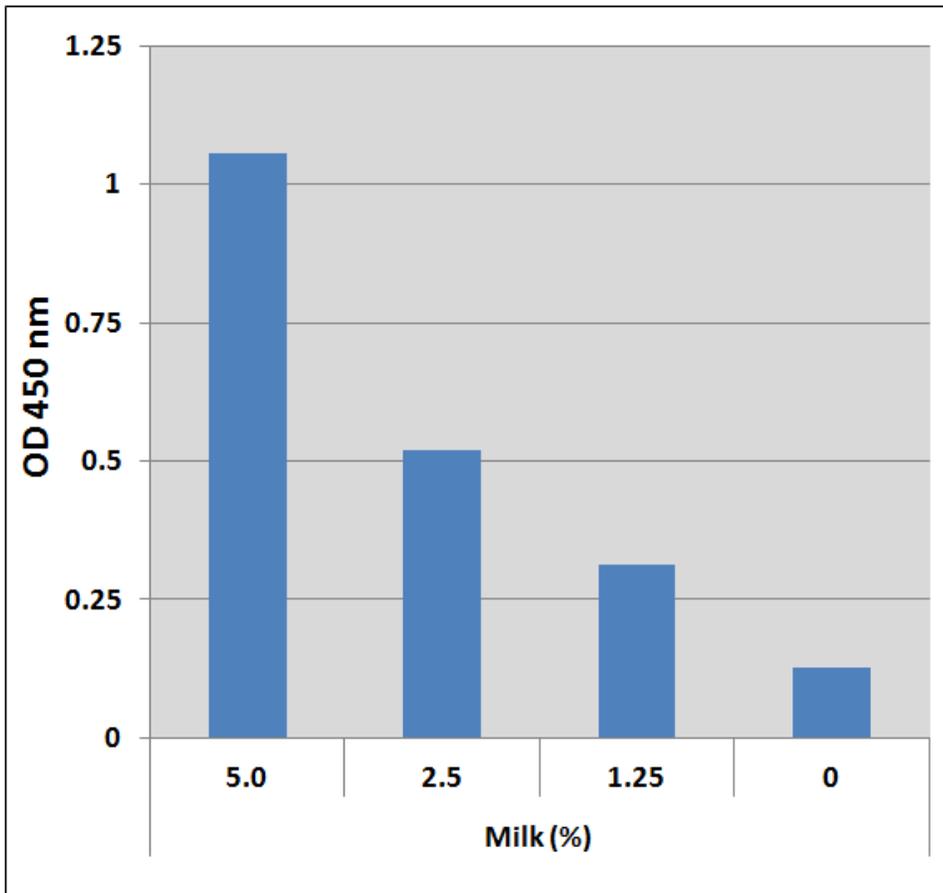


Figure 3: Detection of Histamine in Bovine Milk

References

1. Moon TC, Befus AD, and Kulka M. (2014) *Front. Immun.* **5**: 1-18
2. Ladero V., Calles-Enriquez M., Fernandez M. and Alvarez MA. (2010) *Curr. Nutr & Food Sci.* **6**: 145-156
3. Visciano P., Schirone M., Tofaol R and Suzzi G. (2014) *Front. Microbiol.* **5**:1-3
4. Bruce C, Weatherstone R., Seaton A., and Taylor WH. (1976) *Thorax* **31**:724-729

Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS' sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

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