Histamine Detection Assay

Catalog Number: EZ2024-02

For Research Use Only. Not for use in Diagnostic Procedures.

1. Introduction

Histamine is a pluripotent biogenic amine that acts as both a neurotransmitter and a key immune mediator. In the brain, it regulates wakefulness, appetite, and neuroimmune signaling, while in peripheral tissues it drives inflammation by increasing vascular permeability, contracting smooth muscle, and recruiting immune cells. Released mainly from mast cells, basophils, and certain neurons, histamine influences systems including the respiratory tract, skin, gut, and cardiovascular system. Abnormal histamine signaling contributes to allergy, asthma, anaphylaxis, infection-driven inflammation, and neurological disorders. Accurately measuring and understanding histamine release is essential for advancing important diagnostic and therapeutic strategies. The Attogene Histamine Detection Assay provides a direct and automation-compatible method for measuring histamine levels in samples. The assay utilizes a simple enzymatic reaction to detect histamine. Some unique features of this kit are:

- Simple enzymatic test
- Rapid
- Robust
- Sensitive
- High reproducibility
- Flexible format
- Linearity: 0-20 ppm
- Limit of Quantification (LOQ): 0.5 ppm

Intended use for this kit: For plate-based colorimetric enzymatic determination of histamine content in samples. The kit uses a histamine-specific enzyme to detect histamine directly from samples such as cell culture supernatants and isolated or cultured mast cells.

2. Procedure Overview

After preparing the sample, the assay is performed by adding Histamine Reagent into microplate wells containing 100µL of sample. Following a 10-minute incubation at 25°C, the absorbance of each well at 450nm is measured using a plate reader. The histamine concentration in each sample is then directly determined from the 450nm absorbance, referencing a standard curve. This kit has the capacity for 96 determinations or testing of 40 samples in duplicate (using 16 wells for standards). Store the kit at -20°C. The shelf life is 12 months after receipt when properly stored.

3. Contents (96 determinations)

Component Name	Volumes	Storage
96 well plate	1 each	RT or -20 °C
Histamine Standards: 0ppm, 0.5ppm, 1ppm, 2ppm, 5ppm, 10ppm	0.8 mL x 6	-20 °C
Color Mix	6 mL	-20 °C
Enzyme Mix	6 mL	-20 °C

Required materials not included in kit:

- Microplate reader (wavelength 450 nm)
- 50 mL plastic conical centrifuge tubes
- Centrifuge (for sample preparation)
- Deionized or ultrapure water, if sample dilution is necessary
- Polypropylene microcentrifuge tubes
- Disposable pipet tips and appropriate micropipettes

4. Warnings and Precautions

Attogene strongly recommends that you read the following warnings and precautions to ensure your full awareness of the techniques and other details you should pay close attention to when running the assays. Periodically, optimizations and revisions are made to the kit and manual. Therefore, it is important to follow the protocol coming with the kit.

- Do not use the kit past the expiration date.
- Do not intermix reagents from different kits or different lots.
- Try to maintain a laboratory temperature of 20°- 25°C (68°- 77°F).
- Avoid contamination of reagents. Do not reuse pipet tips.
- Keep containers tightly stoppered.

5. Sample Storage

Be sure samples are properly stored. In general, samples should be refrigerated at 2-4°C for no more than 1-2 days. Freeze samples to a minimum of -20°C if they need to be stored for a longer period. Frozen samples can be thawed at room temps (20 – 25°C / 68 – 77°F) or in a refrigerator before use.

Note: Do not use glassware for extraction purposes. Histamine may adhere to glass which may affect test results. Storage in a polypropylene container is recommended.

6. Histamine Determination Test Protocol

Set-up:

Thaw out frozen kit components before use. Allow thawed components to warm to room temperature for one hour before use. Turn on the plate reader and allow the lamp to warm up. Set the wavelength of the plate reader to 450 nm and the temperature to 25°C.

Reagent Preparation:

All reagents should be mixed by gently inverting or swirling prior to use. Prepare volumes that are needed for the number of wells being run. Do not return the reagents to the original stock tubes/bottles. Using disposable reservoirs when handling reagents can minimize the risk of contamination and is recommended. The thawed reagents can be stored at 4°C for as long as 2 weeks; for a longer time period storage, the reagents should be put in a freezer.

Test Procedure:

1. Preparation of Reaction Mix

Thaw out Color Mix and Enzyme Mix and warm to room temperature (20 – 25°C). Invert tube three times to mix thoroughly. In a clean tube, mix equal parts of the appropriate amounts of Color Mix and Enzyme Mix (1:1, V/V). Use the following table to calculate the required volume for the mixture of these reagents, or the Reaction Mix:

Note: If you are not using the Reaction Mix immediately, store the complete reaction mix on ice or at 4°C until use to prevent background signal.

Reaction Mix (per reaction)		Volume/reaction (total)	24 Reactions	96 Reactions
Color Mix	Enzyme Mix			
50 µL	50 µL	100 µL	2.4 mL	9.6 mL

2. Warm Standards

Warm up the 6 tubes containing Histamine Standards to room temperature (20°- 25°C)

3. Sample Preparation

Reminder: Do not use glassware for extraction purposes. Histamine may adhere to glass which may affect test results. Storage in a polypropylene container is recommended.

Cell Culture Supernatant (Adherant cells)
- Carefully transfer 1 mL of the culture medium to a clean centrifuge tube.
- Centrifuge at 1,000 rpm for 1 minute. The clear supernatant is now ready to be tested.

Cell Culture Supernatant (Suspension cell culture)
- Carefully transfer 1 mL of the culture to a clean centrifuge tube.
- Centrifuge at 1,000 rpm for 1 minute.
- Transfer 0.6 mL of the supernatant to a clean microcentrifuge tube. The clear supernatant is now ready to be tested.

Isolated Mast Cells
- Carefully transfer 1 mL of isolated cells to a clean centrifuge tube.
- Centrifuge at 1,000 rpm for 1 minute.
- Transfer 0.6 mL of the supernatant to a clean microcentrifuge tube. The clear supernatant is now ready to be tested.

Preparation protocols for samples other than above can be made available upon request.

4. Performing the Assay

1. Add 100 µL of each sample or standard (in duplicate) to Microtiter Plate wells, changing tips between samples. It is okay to proceed from low to high standards with the same new tip.

2. Add 100 µL of the Reaction Mix to each well in your assay scheme.

3. Incubate for 10 minutes at room temperature.

4. Measure the absorbance of each well at 450nm in a plate reader.

7. Data Analysis

A standard curve can be constructed by plotting the average corrected absorbance obtained from each reference standard against its concentration in ppm.

Determination of Histamine in Samples:

If the absorbance change in the sample indicates a histamine concentration above the limit of detection and within the linear range, the value can be determined from the standard curve, but if not, the sample in question should be diluted.

Note: The reaction rate can be impacted by temperature; incubating the plate in a set temperature incubator at 25°C can help ensure consistency.

Sample Dilution:

If the test shows the concentration may actually be higher than the highest diluted standard, we recommend carrying out a stepwise dilution of the sample with a histamine-free buffer, such as distilled water, to bring the histamine concentration into the measuring range. The dilution factor must be taken into account when calculating the final histamine content of the sample.

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Our mission is to:

- Enhance detection technologies
- Enable rapid responses
- Enable impactful research discoveries

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