Version 4c, Last updated 7 August 2026

ab235630 Histamine Assay Kit (Colorimetric)

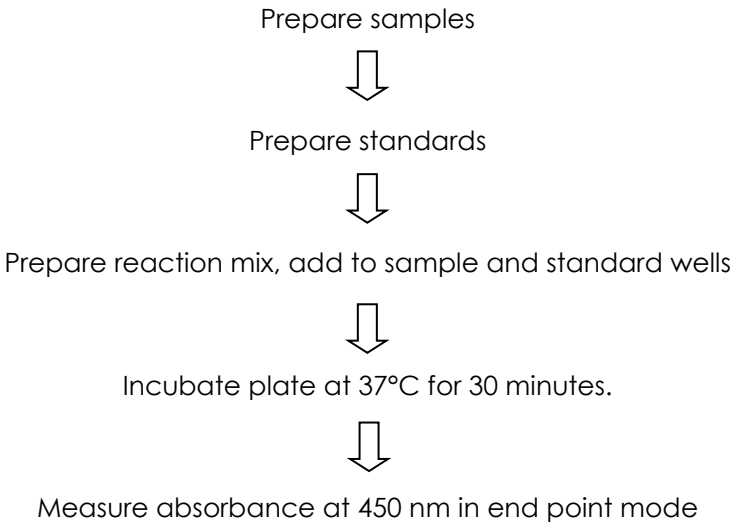
This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview 3
2. Materials Supplied and Storage 4
3. Materials Required, Not Supplied 5
4. General guidelines, precautions, and troubleshooting 6
5. Reagent Preparation 7
6. Standard Preparation 8
7. Sample Preparation 9
8. Assay Procedure 10
9. Data Analysis 11
10. Typical Data 13
11. Notes 13

1. Overview

Histamine is a biogenic amine with considerable biological relevance. As a signalling molecule histamine plays manifold roles in the body, ranging from local immune responses to neurotransmission. Some bacteria generate histamine from histidine via the enzyme histidine decarboxylase.



2. Materials Supplied and Storage

Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components.

Reconstituted components are stable for 2 months.

Aliquot components in working volumes before storing at the recommended temperature.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Histamine Enzyme Mix	1 vial	-20°C	-20°C
Developer Solution III	1 vial	-20°C	-20°C
Histamine Standard	100 µL	-20°C	-20°C
Assay Buffer 63	25 mL	-20°C	-20°C/4°C

PLEASE NOTE: Assay Buffer 63 was previously labelled as Assay Buffer LXIII and Histamine Assay Buffer. The composition has not changed.

3. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at O.D. 450 nm
- 96 well plate with clear flat bottom
- 10 kDa spin column
- 100% methanol
- Dounce homogenizer

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

If applicable, please refer to the current Safety Data Sheet (SDS) provided with this product for safety, handling, and disposal information. The most up to date and current versions are available on our website https://www.abcam.com/en-us.

5. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

5.1 Histamine Enzyme Mix

1. Reconstitute with 220 µL Assay Buffer 63.

5.2 Developer Solution III

1. Bring to room temperature and resuspend in 220 µL Assay Buffer 63.
2. Bring up to room temperature before use.

5.3 Histamine Standard

1. Ready to use as supplied.
2. Bring to room temperature before use.

5.4 Assay Buffer 63

1. Ready to use as supplied.
2. Bring to room temperature before use.

6. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

1. Prepare 1 mM Histamine Standard by adding 10 µL of 50 mM Histamine Standard to 490 µL of ddH_2O.
2. Using 1 mM Histamine Standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard#	1 mM Standard (µL)	Assay Buffer (µL)	Final volume standard in well (µL)	End amount of Histamine standard in well (nmol/well)
1	0	100	50	0
2	2	98	50	1
3	4	96	50	2
4	8	92	50	4
5	12	88	50	6
6	16	84	50	8

Each dilution has enough standard to set up duplicate readings (2 x 50 µL).

7. Sample Preparation

General sample information:

We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.

We recommend that you use fresh samples for the most reproducible assay.

7.1 Liquid Samples:

1. Liquid samples may be assayed directly.

7.2 Solid Samples:

1. Prepare Histamine Sample Buffer by diluting Assay Buffer 63 1:1 with 100% methanol.
2. Homogenize (~200-400 mg) samples using 500 µL Histamine Sample Buffer.

3. Boil sample for 20 minutes at 90°C in sealed tubes, then cool on ice.
4. Centrifuge samples at 10,000 X *g* for 5 minutes.
5. Collect the supernatant.
6. Dilute samples, if necessary, using Assay Buffer 63 and add 5-10 µL into desired well(s) in a 96-well plate.
7. Adjust the volume to 50 µL/well with Assay Buffer 63.

Δ Note: 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 Assay Buffer 63.

Δ Note: For samples having high protein content, we recommend deproteinizing the samples (tissue or cell lysate or biological fluids) using 10 kDa Spin Column (ab93349 or equivalent). Add sample to the spin column, centrifuge at 10,000 x *g*, 4°C for 10 minutes. Collect the filtrate.

Δ Note: 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 un-spiked samples.

8. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.
- Assay all standards, controls and samples in duplicate.

8.1 Reaction mix:

1. Prepare Reaction Mix and Background Mix for each reaction. Prepare a master mix to ensure consistency.

Component	Reaction Mix (µL)	Background Reaction Mix (µL)
Assay Buffer 63	46	48
Histamine Enzyme Mix	2	---
Developer Solution III	2	2

2. Add 50 µL of Reaction Mix into each standard and sample wells.
3. Add 50 µL of Background Reaction Mix into the background control sample wells.
4. Incubate plate at 37°C for 30 minutes.
5. Measure absorbance at 450 nm in end point mode.

9. Data Analysis

Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.

1. Average the duplicate reading for each standard, control and sample.
2. Subtract the mean value of the blank (Standard #1) from all standards, controls and sample readings. This is the corrected absorbance.
3. If significant, subtract the sample background control from sample readings.
4. Plot the corrected values for each standard as a function of the final concentration of Histamine.
5. Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).
6. Apply the corrected sample O.D. reading to the standard curve to get Histamine (B) amount in the sample wells.
7. Concentration of Histamine in nmol/µ/ or mM in the test samples is calculated as:

$$Sample\ Histamine\ concentration = \frac{B}{V} * D$$

Where:

B = amount of Histamine in the sample well calculated from standard curve in nmol.

V = sample volume added in the sample wells µL.

D = sample dilution factor if sample is diluted to fit within the standard curve range (prior to reaction well set up).

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

$$Histamine\ spike\ well\ (B) = \left[\frac{OD\ (sample\ corrected)}{OD(sample + Histamine\ Std\ corrected) - OD\ (sample\ corrected)}\right] * Histamine\ spike\ (nmol)$$

10. Typical Data

Data provided **for demonstration purposes only**.

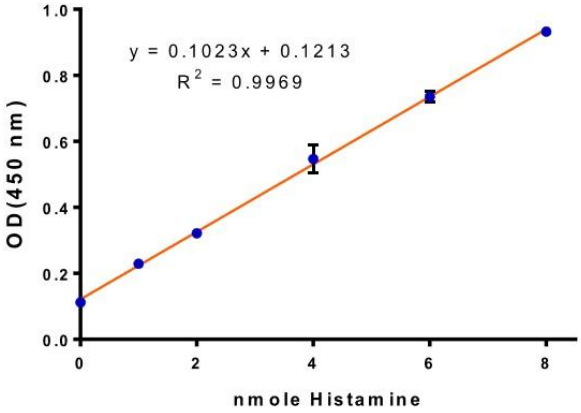


Figure 1. Histamine Standard Curve.

11. Notes

Technical Support

For all technical or commercial enquiries please go to:
https://www.abcam.com/en-us/contact-us
https://www.abcam.cn/contact-us (China)
https://www.abcam.co.jp/contact-us (Japan)