Histamine (Life Science Format) ELISA Kit Instructions

Please read all instructions carefully before beginning this assay

PRODUCT #409010

For research use only.

Storage Conditions:
Do not freeze kit components
All other kit components: 2-8°C

DESCRIPTION

Histamine is a heterocyclic primary amine derived from decarboxylation of the amino acid histidine. It is a mediator of inflammation closely associated with the initial phase of immediate hypersensitivity response (anaphylaxis). Histamine is synthesized by the enzyme histidine decarboxylase and is present in most cells, but typically stored in metachromatic granules of basophils and mast cells (granulocytes) (1). Histamine in the intracellular granules is bound to proteins and inactive until it is released from the cells.

During anaphylactic response, an antigen-IgG antibody complex formed *in vivo* activates the complement cascade and cleaves bioactive complement associated peptides called anaphylatoxins. Among anaphylatoxins, C_{3a}, which is derived from the complement component C_3, and C_{5a} derived from C5 releases histamine from mast cells (2). In IgE-mediated immediate hypersensitivity response, an IgE antibody is produced by B lymphocytes upon stimulation by an allergen and under the control of IL-13 and IL-4. Such IgE antibodies are secreted from B lymphocytes and bound to a high affinity receptor (FceR I High binding IgE receptor) on mast cells in the tissue or on basophilic leukocytes in the peripheral blood leukocytes (3). When IgE bearing mast cells or basophils encounter allergen to which the IgE antibody was directed, the allergen (antigen) binds to the cell bound IgE and agglutinates on the surface of these cells. This event triggers the release of granules into the blood stream. Degranulation of the mast cell involves release of mediators such as leukotriene $C_4/D_4/E_4$, thromboxane A_2, PGD_2, Platelet Activating Factor, histamine, heparin, tryptase, kallikrein, ECF-A, IL-8 and other cytokines. Histamine released from mast cell acts on smooth muscle and blood vessels, causing bronchoconstriction, vasodilation and increased vascular permeability (erythema) (4).

Histamine exerts its biological effects through four distinct receptors on various tissues and cells; H_1, H_2, H_3 and H_4. Among these histamine receptors, H_2 receptor is best recognized as associated with secretion of acid in the stomach leading to peptic ulcer. Thus, an H_2 receptor antagonist is used for treatment of peptic ulcers (5).

Tissue bound mast cells (such as in the skin, nasal mucosa) respond to incoming allergen and manifest as erythema (e.g. in skin test) and wheezing response. In various research areas, it is important to study *in vitro* histamine release from peripheral blood basophils. When whole blood from a sensitized animal is exposed to a given allergen, basophils respond to the allergen by releasing histamine into the incubation mixture. Using a whole blood sample, one can assess ex vivo response to a sensitizing antigen as a function of histamine released from the basophils (6).

Other than histamine being an important mediator of immediate hypersensitivity, histamine is found in decaying fish meat, especially of scombroid fish such as tuna. For this reason histamine is called “Scombrotoxin” (7). Histamine is also found in wine (8) and cheese (9).

INTENDED USE

This kit is designed for *in vitro* quantification of histamine in various biological fluids by competitive direct enzyme-linked immunosorbent assay (CD-ELISA). This kit is intended for use in investigative research only and not for human clinical diagnostic use.

This Histamine ELISA kit (Life Science Format) should not be used for determining histamine levels in scombroid fish. Neogen offers a separate kit for determination of histamine in fish and other foods.

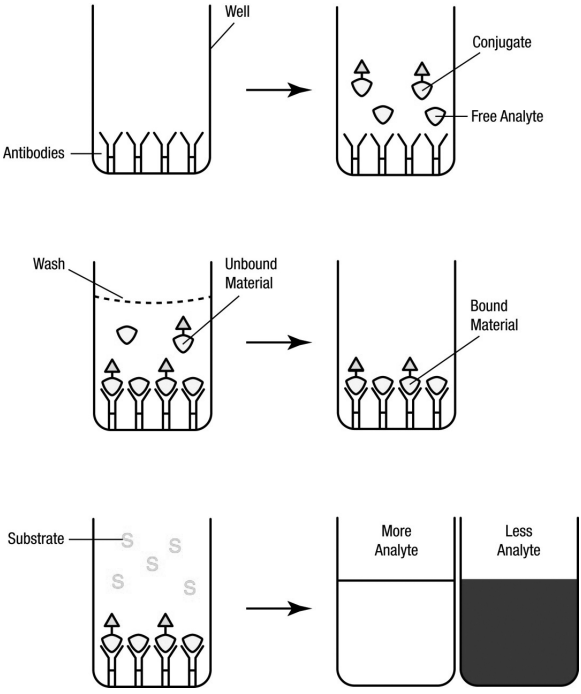
Intended User: Researchers in biomedical fields.

PRINCIPLE OF ASSAY

Neogen's Histamine ELISA test kit (Life Science Format) is a competitive direct ELISA (Enzyme-Linked Immunosorbent Assay) in a microwell format that allows users to obtain exact concentrations of histamine in nanograms per milliliter.

The microwells in this assay kit are pre-coated with a monoclonal antibody to histamine. The sample or standard solution is first added to the antibody coated microplate. Next, the enzyme conjugate is added and the mixture is shaken and incubated at room temperature for 45 minutes. During the incubation, unbound (free) histamine in the samples or standards is allowed to compete with enzyme (horseradish peroxidase: HRP)-labeled histamine (conjugate) for antibody binding sites. The plate is then washed, removing all the unbound material. The bound enzyme conjugate is detected by the addition of a one-component peroxidase substrate which generates color by horseradish peroxidase. An optimal color is generated after 30 minutes. A microplate reader is then used to take an absorbance reading at 650 nm.

Quantitative test results may be obtained by measuring and comparing the absorbance reading of the sample wells against the standard curve using a log-logit curve fitting model. The extent of color development is inversely proportional to the amount of histamine in the sample or standard. For example, the absence of histamine in the sample will result in a bright blue color, whereas the presence of histamine will result in decreased or no color development.



MATERIALS PROVIDED

1. **WASH BUFFER (25X):** 30 mL. Dilute 25-fold with deionized water. Diluted wash buffer is used to wash all unbound enzyme conjugate, samples and standards from the plate after the 45-minute incubation.
2. **K-BLUE® SUBSTRATE:** 20 mL. Stabilized 3,3', 5,5' Tetramethylbenzidine (TMB) plus Hydrogen Peroxide (H_2O_2) in a single bottle. It is used to develop the color in the wells after they have been washed. **LIGHT SENSITIVE**. Keep substrate refrigerated.
3. **PBS SAMPLE DILUENT:** 1 foil pouch of dry powder yields 1 L of 10mM Phosphate Buffered Saline. Once prepared, this buffer is used for diluting extracted and non-extracted samples.
4. **HISTAMINE ENZYME CONJUGATE:** 6 mL. Histamine horseradish peroxidase conjugate, ready-to-use.
5. **HISTAMINE STANDARDS:** 6 vials containing 500 µL per vial. Ready-to-use Histamine standards at the following concentrations: 0, 2.5, 5, 10, 20, 50 ng/mL.
6. **HISTAMINE ANTIBODY-COATED MICROPLATE:** A 96 well Dynex microplate with a monoclonal antibody against Histamine. The plate is ready for use. **DO NOT WASH!**

Storage Requirement: The kit can be used until the expiration date on the label when stored at 2°-8°C.

MATERIALS NEEDED BUT NOT PROVIDED

1. Deionized water for diluting wash buffer and extraction buffer.
2. Precision pipettes that range from 10 µL-1000 µL and disposable tips.

NOTE: If all or several strips are to be used at one time, it is suggested that a multichannel pipette be used.

3. Reagents required for preparation of samples. Since wide varieties of samples can be assayed with this kit, each investigator should use the optimum conditions for extracting histamine with this kit.
4. Disposable reagent boats.
5. Graduated cylinders to dilute and mix wash buffer and extraction buffer.
6. Microplate reader with 650 nm filter.
7. Plate cover or plastic film to cover plate during incubation.

OPTIONAL MATERIALS:

8. 1 N HCl or Neogen's Red Stop Solution (product #301474).
9. Microplate shaker.

WARNINGS AND PRECAUTIONS

1. **DO NOT** use components beyond expiration date.
2. Store test kit between 2-8°C (35-46°F). Avoid prolonged storage of the kit at ambient temperatures. Do not freeze the test kit.
3. Glassware should not be used for extraction purposes. As Histamine may adhere to glass, using glassware may affect test results.
4. The samples to be analyzed with this kit must have a pH of 6-8. Excessively acidic or alkaline samples should be adjusted using 0.1 N NaOH or HCl.
5. **DO NOT** mix any reagents or components of this kit with any reagents or components of any other kit or lot number. This kit is designed to work properly as provided.
6. **DO NOT** pipette reagents by mouth.
7. Use of incubation times other than those specified may give inaccurate results.
8. Always pour substrate out of the bottle into a clean test tube or reagent boat. **DO NOT** pipette out of the bottle. An unclean tip could contaminate the entire contents of the substrate.
9. All specimens should be considered potentially infectious. Exercise proper handling precautions.
10. **DO NOT** smoke, eat or drink in areas where specimens or reagents are being handled.
11. Use aseptic technique when opening and removing reagents from vials and bottles.
12. Keep plate covered except when adding reagents, washing or reading.
13. Kit components should be refrigerated at all times when not in use.

PROCEDURAL NOTES

1. The test kit should be brought to 18-30°C (64-86°F) prior to use.
2. Desiccant bag must remain in foil pouch with unused strips. Keep zip-lock pouch sealed when not in use to maintain a dry environment.
3. Always use new pipette tips to pipette the buffer, enzyme conjugate, standards and samples. This will help to eliminate cross contamination.
4. Before pipetting a reagent, rinse the pipette tip three times with that reagent (i.e. fill the tip with the desired amount of reagent and dispense back into the same vial. Repeat 2 times). Now the tip is properly rinsed and ready to dispense the reagent into your well or test tube.
5. When pipetting into the wells, **DO NOT** allow the pipette tip to touch the inside of the well, or any of the reagents already in the well. This can result in cross contamination.
6. Standards and samples should be assayed in duplicate.
7. To quantitate, always run a standard curve when testing samples.
8. Gently mix specimens and reagents before use. Avoid vigorous agitation.
9. When using only partial amounts of a kit, it is recommended to transfer the appropriate volume of each reagent to a clean vessel for repeated dispensing. This will reduce reagent contamination caused by repeated sampling from the original container.
10. Before taking an absorbance reading, wipe the outside bottom of the wells with a lint-free wiper to remove dust and fingerprints.
11. Before opening the enzyme conjugate and standard vials, tap each vial in an upright position to remove any liquid in the cap.
12. Substrate: Neogen's K-Blue substrate is ready-to-use. Do not dilute. Determine volume needed to perform the test. For each strip of 12 wells, 2 mL of substrate is required or approximately 16 mL of substrate solution is required to run a 96 well microplate. Measure out the required amount of substrate into a clean reagent boat. Do not return unused substrate to the bottle. Keep the substrate protected from light until needed by covering the reagent boat.
13. PBS Sample Diluent: Prepare by adding foil pouch of extract buffer to 1.0 L of deionized or distilled water. Swirl to mix. Store remaining buffer covered at room temperature.

SAMPLE PREPARATION

This assay is non-species specific. The amount of Histamine in your samples may differ. It is recommended that you conduct a preliminary test to determine the optimum dilution for your samples. Typically, tissue culture media, tissue extracts, cell and cell-free extracts can be used.

The samples to be analyzed with this kit must have a pH of 6-8. Excessively acidic or alkaline samples should be adjusted using 0.1 N NaOH or HCl.

TEST PROCEDURES

1. Allow reagents to warm to 18-30ºC (64-86ºF) prior to use.
2. Determine the number of wells to be used.
3. Determine the number of standards to be used. Six standard concentrations are provided with this kit. These standards are ready to use. Neogen recommends using 0, 2.5, 5, 10, 20, 50 ng/mL standards and suggests using a combination of at least 5 standards with each assay.
4. All standards and unknown samples should be assayed in duplicate. See Scheme I for suggested template design.

SCHEME I

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.0	0.0	2.5	2.5	5.0	5.0	10	10	20	20	50	50
B	U1	U1	U2	U2	U3	U3	U4	U4	U5	U5	U6	U6
C	U7	U7	U8	U8	U9	U9	U10	U10	U11	U11	U12	U12
D	U13	U13	U14	U14	U15	U15	U16	U16	U17	U17	U18	U18
E	U19	U19	U20	U20	U21	U21	U22	U22	U23	U23	U24	U24
F	U25	U25	U26	U26	U27	U27	U28	U28	U29	U29	U30	U30
G	U31	U31	U32	U32	U33	U33	U34	U34	U35	U35	U36	U36
H	U37	U37	U38	U38	U39	U39	U40	U40	U41	U41	U42	U42

5. Add 50 µL of standards (S) or unknown (U) (some samples may require diluting) to the appropriate wells in duplicate. Change pipette tips for each standard and unknown solution.
6. Mix each reagent by inverting the reagent bottle prior to use.
7. Add 50 µL of the ready-to-use enzyme conjugate to each well. Use 8-channel pipette or 12-channel pipette for rapid addition.
8. Mix by shaking plate gently. A microplate shaker may be used.
9. Cover the plate with plastic film or plate cover and incubate at room temperature (18-30°C) for 45 minutes. ***NOTE: Keep plate away from drafts and temperature fluctuations.***
10. Dilute concentrated wash buffer with deionized water. The wash buffer is supplied as a 25X concentrate. Prepare by mixing wash buffer concentrate (30 mL) to 720 mL of deionized or distilled water. Swirl to mix. Do not shake. Store remaining wash buffer at room temperature.
11. After the conjugate incubation, dump out the contents of the plate. Tap out contents thoroughly on a clean lint-free towel.
12. Fill each well with 300 µL of the diluted wash buffer and then dump out contents. Repeat this step for a total of three times, then turn the wells upside down and tap out the remaining liquid on a clean lint-free paper towel. If available, an automated plate washer can be used for the wash step. With an automated plate washer, increase the number of wash steps from 3 to 5.
13. Pour the needed volume of substrate from the amber bottle into a reagent boat (not supplied). Add 150 µL of substrate to each well. Use new pipette tips. Neogen recommends using a multichannel pipette for best results. Mix by shaking plate gently. Please note, the remaining substrate in the reagent boat should be discarded.
14. Allow the plate to incubate at room temperature for 30 minutes.
15. Before reading the plate, gently shake the plate by sliding it back and forth on a flat surface. This will ensure uniform color throughout each well. Also use a dry cloth or towel to wipe clean the bottom of the microwells. Air bubbles should be eliminated, as they could affect analytical results.
16. The plate is read in a microplate reader using a 650 nm filter. If a dual wavelength is used, set W_1 at 650 nm and W_2 at 490 nm.
17. If accounting for substrate background, use 2 wells as blanks with only substrate in the wells. (150 µL/well). Subtract the average of these absorbance values from the absorbance values of the wells being assayed.

NOTE: Some microplate readers can be programmed to do these subtractions automatically when reading the plate. Consult your instrument manual.

18. If the microplate can not be read after the 30 minute substrate incubation, add 50-100 µL of 1 N HCl or Neogen's Red Stop Solution to each well to stop enzyme reaction.
19. Read plate at 450 nm, if 1 N HCl solution was used to stop the reaction. Read plate at 650 nm if Neogen's Red Stop Solution was used. If a dual wavelength is used, set at W_1 450 nm and at W_2 650 nm.
20. Plot the standard curve and estimate the concentrations of the samples from the curve. See "CALCULATIONS."

Note: Absorbance readings will approximately double when stopped with acid. If absorbance readings are too high for measuring with your microplate reader, decrease the substrate incubation from 30 minutes to 15 or 20 minutes.

CALCULATIONS

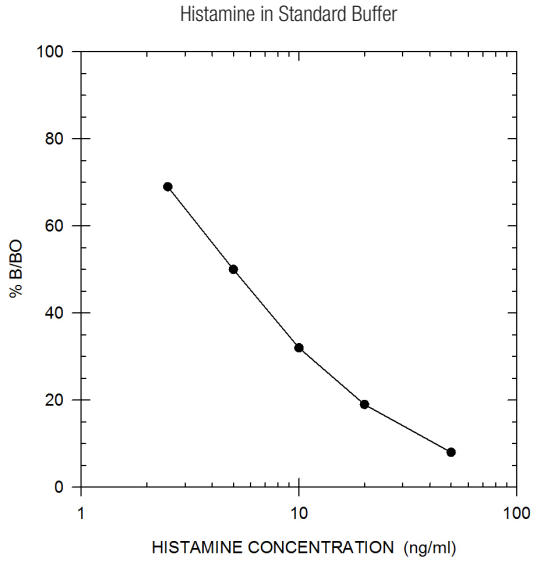
1. A log-logit curve fitting model for calculating the histamine levels of unknown samples is recommended.
2. Subtract the average substrate background absorbance from all standard and sample absorbance values. Average duplicate well absorbance values.
3. Calculate the percent of maximal binding (%B/B_0 value) of each standard by dividing the absorbance of the standard by the 0 standard absorbance and multiply by 100. Transform the ratio into the logit function, where logit = ln (%B/B_0/(100-%B/B_0)).

   Example: 0 Standard Absorbance = 1.800 = B_0
   10 ng/mL Standard Absorbance = 1.000
   %B/B0 = 1.000/1.800 x 100 = 55.5
   Logit = ln(55.5/(100-55.5) = 0.221
4. Repeat for all of the standards.
5. Graph your standard curve by plotting the logit for each standard concentration on the ordinate (y) axis against the log of the standard concentrations on the abscissa (x) axis. Draw a curve by using a curve-fitting routine (i.e. 4-parameter or linear regression). A log-logit curve is recommended for this assay.
6. Determine the %B/B_0 and logit values for each sample:

   Example: 0 Standard Absorbance = 1.800 = B_0
   10 ng/mL Standard Absorbance = 0.600
   %B/B_0 = 0.600/1.800 x 100 = 33.3
   Logit = ln(33.3/(100-33.3) = -0.695
7. Using the standard curve, the concentration of each sample can be determined by comparing the logit value of each sample to the corresponding concentration of Histamine standard.
8. If the samples were diluted, the concentration determined from the standard curve must be multiplied by the dilution factor. If the absorbance values of a sample fall outside the values of the 2.5 or 50 ng/mL standard, concentrate or dilute the sample as appropriate and retest.
9. To convert mass based concentration of Histamine into molarity the following equation can be used: ng/mL x 9.005 = nmole/L (nM). E.g. 1.0 ng/mL = 9 nM.

TYPICAL STANDARD CURVE

A typical standard curve expressed as log-logit format is shown below.



TYPICAL DATA

NOTE: "Typical data" is a representation. Variances in data will occur. Optical density readings may fluctuate during the shelf-life of the kit, but the %B/B_0 should remain comparable. Measuring wavelength: 650 nm

Standard	*Standard Concentration (ng/mL)*	*Optical Density (Absorbance Value)*	*%B/B_0*
S_0 (B_0)	0	2.186	100
S_1 (B_1)	2.5	1.498	69
S_2 (B_2)	5.0	1.089	50
S_3 (B_3)	10	0.700	32
S_4 (B_4)	20	0.413	19
S_5 (B_5)	50	0.185	8

PERFORMANCE CHARACTERISTICS

Limit of quantification: 2.5 ng/mL. Described as the lowest concentration point on the calibration curve that this test can reliably detect Histamine.

Range of quantification: 2.5 - 50.0 ng/mL. For quantitating samples above 50.0 ng/mL, contact Neogen Technical Services for dilution instruction if required.

Intra-assay Precision: ≤ 10%

Inter-assay Precision: ≤ 10%

Validated matrix: No application is developed for use with tissue culture, cell extracts, etc.

CROSS REACTIVITY

The monoclonal antibody used in Neogen's Histamine Kit is highly specific to Histamine as shown below.

HISTAMINE 100.0%
HISTIDINE ..... <0.01%
CADAVERINE ..... <0.01%
TYRAMINE ..... <0.01%
SPERMINE ..... <0.01%
PUTRESCINE ..... <0.01%
TRIMETHYLAMINE ..... <0.01%

DISCUSSION

Histamine release reactions *in vivo* and *in vitro* are investigated by various researchers. Some investigators use HPLC with fluorimetric detector, radioimmunoassay, and enzyme immunoassay to determine histamine contents of biological fluids. One attractive feature of studying histamine release using ELISA is that one can use whole blood to activate cells with stimulants and measure histamine in the same reaction mixture. The normal plasma level of histamine is less than 1 ng/mL, and 3-7 ng/mL is found in animals or patients with allergic response. Histamine contents of whole blood from human are between 20 to 200 ng/mL. In clinical situations, arterial hypotension is observed in patients whose plasma histamine reached 6 –8 ng/mL, bronchospasm at 7 – 12 ng/mL. If plasma histamine exceeds 100 ng/mL, it is lethal. Animal and fish tissues contain 1 –100 µg/g tissue.

Ferrer et al (10) showed that histamine can be released from whole blood of patients in response to antigenic response. Histamine can also be released from mouse mast cell line. Histamine release is modulated by addition of tetracosahexaenoic acid in the culture media (11). Eugenol (a major component of clove) reduced Compound 48/80-induced systemic anaphylaxis in rat. Eugenol also inhibited cutaneous anaphylaxis in response to anti-DNP-IgE and reduced serum histamine levels (12). Demoly et al used histamine release to predict allergic response to therapeutic drugs (13). In this paper, drug specific histamine release from venous blood (whole blood) was compared with the total histamine released by freeze-thawing the cells. The total histamine release by freeze-thawing was 61 ng/mL (median value).

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TECHNICAL ASSISTANCE

Technical assistance is available Monday-Friday, between 8:00 a.m. and 6:00 p.m. EST.

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