Enzyme Immunoassay for
Histamine (Life Science Format)

For Research Use Only

INTRODUCTION

Histamine is a heterocyclic primary amine derived from the 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, C3a, which is derived from the complement component C3, and C5a 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 an 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 the release of mediators such as leukotriene C_4, D_4, B_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 three distinct receptors on various tissues and cells: H1, H2 and H3. Among these histamine receptors, H2 receptor is best recognized as associated with secretion of acid in the stomach leading to peptic ulcer. Thus, an H2 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).

PRINCIPLES OF PROCEDURE

This Histamine kit 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 substrate that 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 with the use of 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.

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 kit (Life Science Format) should not be used for determining histamine levels in scombroid fish.

Intended User: Researchers in biomedical fields.

MATERIALS PROVIDED

Component	Description	Volume	Storage
PBS Buffer	Buffer used to dilute extracted and non-extracted samples.	1 pouch	4°C
25x Wash Buffer	Buffer used to wash the plate prior to color development.	30 mL	4°C
TMB Substrate	TMB substrate used for color development.	20 mL	4°C
Histamine Conjugate	Histamine horseradish peroxidase conjugate (ready-to-use).	6 mL	4°C
Histamine Standards	6 vials of Histamine standard solutions at 0, 2.5, 5, 10, 20, 50 ng/mL.	500 μL	4°C
Coated Plate	96-well microplate coated with monoclonal anti-Histamine antibody.	1 plate	4°C

MATERIALS NEEDED BUT NOT PROVIDED

1. Microplate reader with a 450 nm or 650 nm filter
2. Adjustable micropipettes (10 – 1000 μL) and tips
3. Deionized water
4. Plate cover or plastic film
5. 1 N HCl (optional)

STORAGE

1. Store the components of this kit at the temperatures specified on the labels.
2. Unopened reagents are stable until the indicated kit expiration date.
3. Desiccant bag must remain in foil pouch with unused strips. Keep pouch sealed when not in use to maintain a dry environment. Remove excess air before sealing.

WARNINGS AND PRECAUTIONS

1. Use aseptic technique when opening and dispensing reagents.
2. This kit is designed to work properly as provided and instructed. Additions, deletions or substitutions to the procedure or reagents are not recommended, as they may be detrimental to the assay.

PROCEDURAL NOTES

1. Glassware should not be used for extraction purposes. As Histamine may adhere to glass, using glassware may affect test results.
2. The kit should be brought to room temperature prior to use.
3. To minimize errors in absorbance measurements due to handling, wipe the exterior bottom of the microplate wells with a lint-free paper towel prior to inserting into the plate reader.

SAMPLE PREPARATION

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.

REAGENT PREPARATION

1. **PBS Buffer:** Add the contents of the pouch to 1.0 L of deionized water prior to use.
2. **25x Wash Buffer:** Add 30 mL to 720 mL of deionized water prior to use.

STANDARD CURVE PREPARATION

The Histamine Standards are provided ready-to-use in the following concentrations: 0, 2.5, 5, 10, 20, and 50 ng/mL.

ASSAY PROCEDURE

1. Add 50 µL of Standards or Samples (may require diluting) to the corresponding wells on the microplate in duplicate. See **Scheme I** for a sample plate layout.
2. Add 50 µL of Histamine-HRP Conjugate to each well. Incubate at room temperature for 45 minutes.
3. Wash the plate three times with 300 µL of diluted Wash Buffer per well. Wash 5 times if using an automated plate washer.
4. Add 150 µL of TMB Substrate to each well. Incubate at room temperature for 30 minutes.
5. Read the plate at 650 nm.
   Alternately, the color reaction can be stopped after 15-20 minutes by adding 50 µL of 1 N HCl and read at 450 nm.
   NOTE: If accounting for substrate background, use 2 wells as blanks (BLK) with only 150 µL TMB Substrate in the wells. Subtract the average of these absorbance values from the absorbance values of the wells being assayed.

Scheme I: Sample Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	S_0	S_0	U_3	U_3	U_{11}	U_{11}	U_{19}	U_{19}	U_{27}	U_{27}	U_{35}	U_{35}
B	S_1	S_1	U_4	U_4	U_{12}	U_{12}	U_{20}	U_{20}	U_{28}	U_{28}	U_{36}	U_{36}
C	S_2	S_2	U_5	U_5	U_{13}	U_{13}	U_{21}	U_{21}	U_{29}	U_{29}	U_{37}	U_{37}
D	S_3	S_3	U_6	U_6	U_{14}	U_{14}	U_{22}	U_{22}	U_{30}	U_{30}	U_{38}	U_{38}
E	S_4	S_4	U_7	U_7	U_{15}	U_{15}	U_{23}	U_{23}	U_{31}	U_{31}	U_{39}	U_{39}
F	S_5	S_5	U_8	U_8	U_{16}	U_{16}	U_{24}	U_{24}	U_{32}	U_{32}	U_{40}	U_{40}
G	U_1	U_1	U_9	U_9	U_{17}	U_{17}	U_{25}	U_{25}	U_{33}	U_{33}	U_{41}	U_{41}
H	U_2	U_2	U_{10}	U_{10}	U_{18}	U_{18}	U_{26}	U_{26}	U_{34}	U_{34}	BLK	BLK

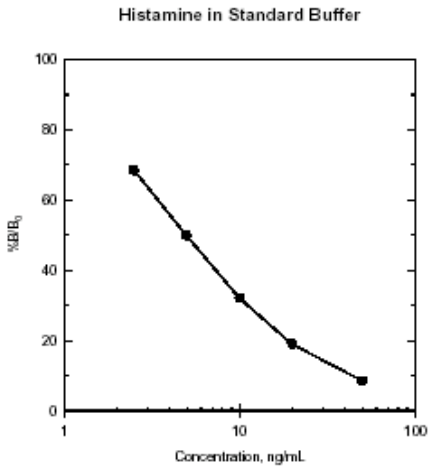
CALCULATIONS

1. After the substrate background has been subtracted from all absorbance values, average all of your duplicate well absorbance values.
2. The average of your two S_0 values is now your B_0 value. (S_1 now becomes B_1, etc.)
3. Next, find the percent of maximal binding (%B/B_0 value). To do this, divide the averages of each standard absorbance value (now known as B_1 through B_7) by the B_0 absorbance value and multiply by 100 to achieve percentages. Transform the ratio into a logit function using the following equation:

$$Logit = \ln\left(\frac{(\%B / \%B_0)}{100 - (\%B / \%B_0)}\right)$$

4. Graph your standard curve by plotting the logit for each standard concentration on the y-axis against the log of the standard concentrations on the 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.
5. Find the percent of maximal binding and the logit function of each sample.
6. Using the standard curve, the concentration of each sample can be determined by comparing the %B/B_0 of each sample to the corresponding concentration of histamine standard.
7. If the samples were diluted, the concentration determined from the standard curve must be multiplied by the dilution factor.
8. 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.

Figure 1: Typical Standard Curve



CROSS REACTIVITY

Histamine	100.0%	Spermine	<0.01%
Histidine	0.008%	Putrescine	<0.01%
Cadaverine	0.003%	Trimethylamine	<0.01%
Tyramine	<0.01%		

PERFORMANCE CHARACTERISTICS

Limit of quantification: 2.5 ng/mL.
Range of quantification: 2.5 - 50.0 ng/mL.
Intra-assay Precision: < = 10%
Inter-assay Precision: < = 10%

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 humans 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 the 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 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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