Histamine ELISA Assay Kit

Catalog Number:
HIS31-K01

For Research Use Only. Not for use in diagnostic procedures.
v. 1.0

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INTENDED USE

The Eagle Biosciences Histamine ELISA Assay Kit is intended for the quantitative determination of histamine in Plasma, Urine, and Cell Culture. The Histamine ELISA Kit is for research use only and not to be used in clinical, therapeutic or diagnostic procedures.

INTRODUCTION

Histamine (β-imidazole-ethylamine) a biogenic amine, is a product of the histidine metabolism. It is produced by decarboxylation of histidine.
Histamine is widely distributed in mammalian tissues. It's bound to heparin (as inactive form) and stored in the granules of basophilic leukocytes and mast cells and is actively released as required. These cells, if sensitized by IgE antibodies attached to their membranes, degranulate when exposed to the appropriate antigen.
Histamine plays a major rule in the initial phase of an anaphylactic reaction.
The quantification of histamine in plasma after allergen administration is of clinical interest.
Histamine is part of the immune response to foreign pathogens and it increases the permeability of the capillaries to white blood cells and other proteins, in order to allow them to engage foreign invaders in the affected tissues. Responsible for the biological effects of histamine in tissue are the activation of different surface receptors, for instance H1, H2 and H3.
Histamine is involved in the regulating physiological function in the gut and acting as a neurotransmitter.

PRINCIPLE OF THE ASSAY

The competitive Histamine ELISA kit uses the microtitre plate format. Histamine is bound to the solid phase of the microtiter plate. Acylated histamine and solid phase bound histamine compete for a fixed number of antiserum binding sites. When the system is in equilibrium, free antigen and free antigen-antiserum complexes are removed by washing. The antibody bound to the solid phase histamine is detected by anti-rabbit/peroxidase. The substrate TMB / peroxidase reaction is monitored at 450 nm. The amount of antibody bound to the solid phase histamine is inversely proportional to the histamine concentration of the sample.

PRECAUTIONS

- For research use only
- Disposable gloves and safety glasses should be used.
- All reagents of human origin used in this kit are tested for HIV I/II antibodies, HCV and HBsAg and found to be negative. However, because no test method can offer complete assurance that infectious agents are absent, these reagents should be handled as potentially biohazardous materials.
- Material of animal origin used in the preparation of the kit have been obtained from certified healthy animals but these materials should be handled as potentially infectious.
- Some components of this kit are containing hazardous reagents. These components are marked with the adequate hazard label.

STORAGE AND STABILITY

On arrival, store the kit at 2-8 °C. Once opened the kit is stable until its expiry date. For stability of prepared reagents refer to Preparation of Reagents.
Do not use components beyond the expiration date shown on the labels.
Do not mix various lots of any kit component within an individual assay.

CONTENTS OF THE KIT

4.1 MT-Strips 12 strips
8 wells each, break apart, precoated with:
Derivatized dopamine (12 strips), colour coded green

4.2 Standards (1-6) 6 vials
Each 4 ml, ready for use
Concentrations:

Standard	1	2	3	4	5	6
Ng/ml	0	0.2	0.6	2	6	25

4.3 Control 1&2 2 vials
Each 4 ml, ready for use
Concentrations: see q.c. certificate

4.4 Acylation Buffer 1 vial
6 ml, ready for use
Colour coded blue

4.5 Acylation Reagent 3 vials
Lyophilized, dissolve content
In 1.5 ml

4.6 Solvent 1 vial
5.5 ml solvent to dissolve the Acylation reagent
Contains acetone, ready for use

4.7 Antiserum 1 vial
5.5 ml, ready for use, colour coded yellow
Rabbit-anti-N-acyl-histamine

4.8 Enzyme Conjugate 1 vial
12 ml, ready for use
Goat anti-rabbit-IgG-peroxidase

4.9 Wash Buffer 1 vial
20 ml, 50x concentrated
Dilute content with distilled water to 1 litre total volume

4.10 Substrate 1 vial
12 ml TMB solution, ready for use

4.11 Stop Solution 1 vial
12 ml, ready for use
Contains 0.3 M sulphuric acid

4.12 Reaction Plate 2 plates
For acylation

4.13 Equalizing Reagent 1 vial
Lyophilized, dissolve contents with distilled water,
Volume: see vial label

ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

- Pipettes 20, 30, 50, and 100 µl
- Orbital shaker
- Multichannel pipette or Microplate washing device
- Microplate photometer (450 nm)
- Distilled water

SAMPLE COLLECTION AND STORAGE

The test can be performed with EDTA or Heparin plasma, urine and cell culture media. Repeated freezing and thawing of samples should be avoided.

Plasma

EDTA or Heparin plasma can be used. Haemolytic and lipaemic samples should not be used. The samples can be stored up to 6 hours at 2 - 8 °C. For a longer storage (up to 6 months) the samples must be frozen at -20 °C

Urine

Spontaneous urine can be used for this test as well as collected urine. In this case the total volume of urine excreted during a 24-hours period should be collected and mixed in a single bottle containing 10 - 15 ml of 6 M hydrochloric acid as preservative. Avoid exposure to direct sun light. Determine the total volume and take an aliquot for the measurement. For patients with suspected kidney disorders the kreatinine concentration should be tested, too. Urine samples can be stored at -20 °C for at least 6 months.
Urine samples have to be diluted 1:15 with dist. water before assay.

Cell Culture Media

Media like DMEM and RPMI can be used in the test. Other media have to be checked by the user.

PREPARATION OF REAGENTS AND SAMPLES

Preparation of Reagents

Wash Buffer

Dilute the content with dist. water to a total volume of 1,000 ml.
The diluted wash buffer has to be stored at 2 - 8 □ C for a maximum period of 4 weeks. For longer storage freeze at -20 °C.

Equalizing Reagent

Dissolve the contents with dist. water (for volume refer to vial label), mix shortly and leave on a roll mixer for minimum 20 minutes. Handle with care in order to minimize foam formation. The reconstituted Equalizing Reagent should be stored frozen at -20 °C and is stable until expiry date printed on vial label.

Acylation Reagent
Dissolve the content of one bottle in 1.5 ml Solvent and shake for 5 minutes on an orbital shaker. The Acylation Reagent has always to be prepared immediately before use. After use the reagent has to be discarded.
The second and third vial allows a second and third run of the test, respectively. If the whole kit is to be used in one run it is recommended to pool the dissolved contents of the three vials of Acylation Reagent.
Please note that solvent reacts with many plastic materials including plastic trays; solvent does not react with normal pipette tips and with glass devices
Solvent is volatile and the dissolved Acylation Reagent evaporates quickly. Therefore, please do not use a tray with big surface together with a multichannel pipette for pipetting Acylation Reagent. Rather, use an Eppendorf multipette (or similar device), fill the syringe directly from the vial with dissolved Acylation Reagent and add well by well.

All other reagents are ready for use.

Preparation of Samples (Acylation)

Allow reagents and samples to reach room temperature.
Determinations in duplicates are recommended.
The wells of the reaction plate for the acylation can be used only once. So please mark the respective wells before using.

1. Pipette each 50 µl standard 1 - 6, 50 µl control 1 & 2, 50 µl EDTA plasma samples, 20 µl Heparin plasma, 50 µl urine samples (diluted 1:15 with dist. water) and 50 µl cell culture media samples into the respective wells of the reaction plate.
2. Pipette each 50 µl Acylation Buffer into all wells.
3. Pipette 50 µl dist. water in all wells containing plasma samples.
4. Pipette each 50 µl Equalizing Reagent into wells containing standards, controls, urine samples and cell culture media samples. Pipette each 30 µl Equalizing Reagent into wells containing Heparin plasma. Do not pipette into wells containing EDTA plasma samples. Mix the reaction plate for 10 seconds.
5. Pipette each 10 µl Acylation Reagent into all wells and continue with step 6. immediately. Colour changes to violet.
   Please note that solvent reacts with many plastic materials including plastic trays; solvent does not react with normal pipette tips and with glass devices
   Solvent is volatile and the dissolved Acylation Reagent evaporates quickly. Therefore, please do not use a tray with big surface together with a multichannel pipette for pipetting Acylation Reagent. Rather, use an Eppendorf multipette (or similar device), fill the syringe directly from the vial with dissolved Acylation Reagent and well by well.
6. Incubate for 15 minutes at room temperature on an orbital shaker with medium frequency. Do not cover the wells or the plate; leave the plate open on the shaker.
7. Pipette 50 µl Antiserum into all wells.
8. Incubate for 30 minutes at room temperature on an orbital shaker with medium frequency. Do not cover the wells or the plate; leave the plate open on the shaker.

   Take each 50 µl for the ELISA.

TEST PROCEDURE ELISA

Allow reagents and samples to reach room temperature.
Determinations in duplicates are recommended.

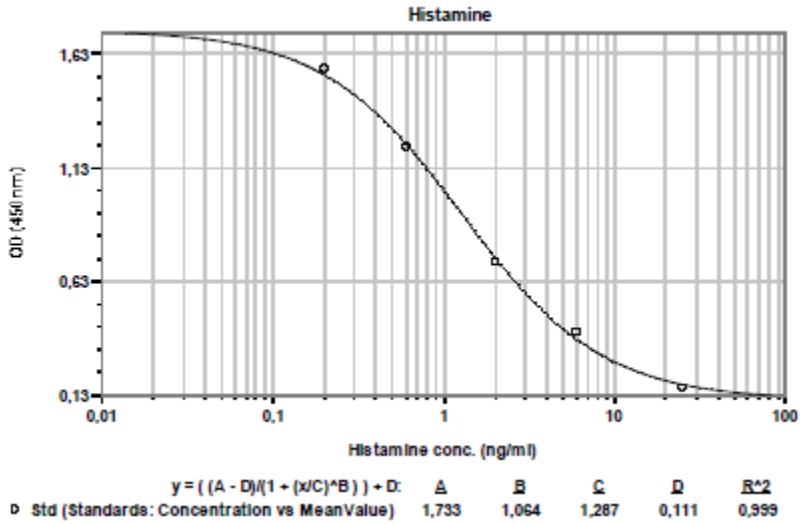
1. Pipette each 50 µl prepared Standards 1 to 6, Controls and Samples into the respective wells of the coated microtiter strips.
2. Incubate for 60 minutes at room temperature (20 – 25 °C) on an orbital shaker with medium frequency. Alternative: Shake the plate briefly manually and incubate 90 minutes without shaking.
3. Discard or aspirate the contents of the wells, add each 300 µl Wash Buffer, again discard or aspirate the contents of the wells. Remove residual liquid by tapping the inverted plate on clean absorbent paper.
   Repeat the washing procedure 4 times.
4. Pipette each 100 µl enzyme conjugate into all wells.
5. Incubate for 20 minutes at room temperature on an orbital shaker with medium frequency. Alternative: Shake the plate briefly manually and incubate 25 minutes without shaking
6. Washing: Repeat step 3.
7. Pipette each 100 µl Substrate into all wells.
8. Incubate for 20 ± 5 minutes at room temperature (20 - 25 °C) on an orbital shaker with medium frequency. Alternative: Shake the plate briefly manually and incubate 20 ± 5 minutes without shaking
9. Pipette each 100 µl Stop Solution into all wells.
10. Read the optical density at 450 nm (reference wavelength between 570 and 650 nm) in a microplate photometer within 15 minutes.

CALCULATION OF THE RESULTS

On a semilogarithmic graph paper the concentration of the standards (x-axis, logarithmic) are plotted against their corresponding optical density (y-axis, linear). Alternatively, the optical density of each standard and sample can be related to the optical density of the zero standard, expressed as the ratio OD/ODmax, and then plotted on the y-axis. Evaluation by 4 parameter iteration or cubic spline is recommended.
The concentration of the controls and plasma samples and cell culture media can be read directly from this standard curve by using their average optical density.
The read concentration of Heparin plasma samples has to be multiplied by a factor of 2.5.
The read concentration of urine samples has to be multiplied by a factor of 15.

Conversion: 1 ng/ml corresponds to 9,0 nmol/l

Typical standard curve:



ASSAY CHARACTERISTICS

Normal Range

EDTA Plasma	< 1 ng/ml
Heparin Plasma	< 4.5 ng/ml
Urine	<45 µg/day

The reference range given below should only be taken as a guideline. It is recommended that each laboratory should establish its own normal values.

Sensitivity

The lower limit of detection was determined by taking the 2fold standard deviation of the absorbance of the Zero Reference and reading the corresponding value from the standard curve.

	Sensitivity
EDTA Plasma	0.06 ng/ml
Heparin Plasma	0.15 ng/ml
Urine	0.9 ng/ml

Specificity (Cross Reactivity)

Structural related components were tested for possible interference with the antisera against histamine used in the ELISA method.

Substance	**Cross Reactivity (%)**
Histamine	100
1-methyl histamine	0.054
3-methyl histamine	0.13
1-methyl-4-imidazol-acetic acid	<0.0001

Imidazol-4-acetic acid	<0.0002
L-histadine	<0.0001

Recovery
Increasing amounts of histamine were added to each sample. Each spiked sample was assayed. The analytical recovery of histamine was estimated at different concentrations by using the theoretically expected and the actually measured values.
Concentrations in ng/ml

	Range (ng/ml)	**Mean (%)**	**Range (%)**
EDTA Plasma	0.6-13.4	101	93-111
Heparin Plasma	0.8-36.0	104	87-112
Urine	6.1-140.6	98	94-103
Cell Culture Media	1.0-12.9	104	91-121

Linearity
The linearity of the ELISA method was investigated using different dilutions of a sample.
Concentrations in ng/ml

	Range (ng/ml)	**Highest Dilution**	**Mean (%)**	**Range (%)**
EDTA Plasma	0.5-10.0	1:20 Equalizing Reagent	106	96-111
Heparin Plasma	0.9-13.7	1:15 Equalizing Reagent	102	93-109
Urine	7-142	1:20 dist. water	96	81-102
Cell Culture Media	1.1-10.3	1:10 dist. water	106	99-11

Reproducibility
The reproducibility of the ELISA method was investigated by measuring the intra- and inter-assay-coefficients of variation (cv).
Concentrations in ng/ml

Sample	Range (ng/ml)	Intra-Assay cv %	Range (ng/ml)	Inter-Assay cv %
EDTA-Plasma	1.2-8.7	6.1-6.5	1.1-3.3	6.2-7.3
Heparin Plasma	2.5-11.8	6.3-5.0	2.1-10.8	8.9-4.4
Urine	24.1-89.6	6.6-5.7	15.7-43.9	7.2-11.3
Cell Cutlure	1.5-5.1	6.3-8.6	1.3-4.1	10.5-6.5

LITERATURE

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Pipetting Scheme Sample Preparation

		Standards	Control	EDTA Plasma	Heparin Plasma	Urine (dil.)	Medium
Standard 1 - 6	µl	50					
Control 1 & 2	µl		50				
EDTA Plasma	µl			50			
Heparin Plasma	µl				20		
Urine (1:15 dil.)	µl					50	
Medium	µl						50
Acyl. Buffer	µl	50	50	50	50	50	50
Dist. Water	µl			50	50		
Equalizing Reag.	µl	50	50		30	50	50

shake for 10 seconds

Acyl. Reagent	µl	10	10	10	10	10	10

Immediately shake 15 minutes at room temperature, leave plate open

Antiserum	µl	50	50	50	50	50	50

shake 30 minutes at room temperature, leave plate open

take each 50 µl for the ELISA

Pipetting Scheme ELISA

		Standards	Control	Sample
Standard 1 - 6	µl	50		
Control 1 & 2	µl		50	
Acyl. Sample	µl			50

shake for 60 minutes at room temperature

4 x washing

Enzyme Conjugate	µl	100	100	100

shake for 20 minutes at room temperature

4 x washing

Substrate	µl	100	100	100

shake for 15 - 25 minutes at room temperature

Stop Solution	µl	100	100	100

Reading of absorbance at 450 nm

Warranty Information

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For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.