



Instruction for use


Histamine ELISA


Enzyme Immunoassay
for the Quantitative Determination of
Histamine in Plasma, Urine and Cell Culture Media

CE

IVD

REF EA213/96

 12 x 8

 2 – 8 °C

DLD Gesellschaft für Diagnostika und medizinische Geräte mbH
Adlerhorst 15 • 22459 Hamburg • Germany
Tel +49-40-555 87 10 • Fax +49-40-555 87 111
Internet: <http://www.dld-diagnostika.de> • E-Mail: contact@dld-diagnostika.de

Contents

1. Introduction and Principle of the Test	Page	5
2. Precautions	Page	6
3. Storage and Stability	Page	6
4. Contents of the Kit	Page	6
5. Specimen Collection and Storage	Page	8
6. Preparation of Reagents and Samples	Page	9
7. Test Procedure (ELISA)	Page	11
8. Calculation of the Results	Page	12
9. Assay Characteristics	Page	13
10. Literature	Page	15
Pipetting Scheme	Page	16

1. Introduction and Principle of the Test

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 role 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.

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.

2. Precautions

- For in vitro 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.

3. 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 kit labels. Do not mix various lots of any kit component within an individual assay.



4. Contents of the Kit

4.1 **MT-Strips** **STRIPS** 12 strips
8 wells each, break apart
precoated with histamine

4.2 **Standards 1 - 6** **CAL 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** **CON 1 & 2** 2 vials
Each 4 ml, ready for use
Range: see q.c. certificate

4.4	Acylation Buffer 6 ml, colour coded blue, ready for use	ACYL-BUFF	 1 vial
4.5	Acylation Reagent lyophilised, dissolve content in 1.5 ml Solvent	ACYL-REAG	3 vials
4.13	Solvent 5.5 ml solvent to dissolve the Acylation reagent Contains acetone, ready for use	SOLVENT	 1 vial
4.6	Antiserum 5.5 ml, ready for use, colour coded yellow rabbit-anti-N-acyl-histamine	AS	1 vial
4.7	Enzyme Conjugate 12 ml, ready for use goat anti-rabbit-IgG-peroxidase	CONJ	1 vial
4.8	Wash Buffer 20 ml, 50x concentrated Dilute content with distilled water to 1 litre total volume	WASH	1 vial
4.9	Substrate 12 ml TMB solution, ready for use	SUB	1 vial
4.10	Stop Solution 12 ml, ready for use contains 0.3 M sulphuric acid	STOP	1 vial
4.11	Reaction Plate for acylation	ACYL-PLATE	2 plates
4.12	Equalizing Reagent lyophilized, dissolve contents with distilled water, volume: see vial label	EQUA-REAG	1 vial
4.15	Adhesive Foil Ready for use	Foil	1 piece

Additional materials and equipment required but not provided:

- Pipettes 50 and 100 μ l
- Orbital shaker
- Multichannel pipette or Microplate washing device
- Microplate photometer (450 nm)
- Distilled water

5. Specimen Collection and Storage

The test can be performed with EDTA plasma, urine and cell culture media.

Repeated freezing and thawing of samples should be avoided.

EDTA Plasma

EDTA plasma should 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.

6. Preparation of Reagents and Samples

6.1. Preparation of Reagents

Wash Buffer

WASH

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

EQUA-REAG

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

ACYL-REAG

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 multipipette (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.

6.2. 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 μ l standard 1 - 6, control 1 & 2 and plasma samples, urine samples (diluted 1:15 with dist. water) and cell culture media samples into the respective wells of the reaction plate.
2. Pipette each 50 μ l Acylation Buffer into all wells.
3. Pipette 50 μ l dist. water in all wells containing plasma samples.
4. Pipette each 50 μ l Equalizing Reagent into wells containing standards, controls, urine samples and cell culture media samples. Do not pipette into wells containing plasma samples. Mix the reaction plate for 10 seconds.
5. Pipette each 10 μ l 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 multipipette (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 μ l 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 μ l for the ELISA.

7. Test Procedure ELISA

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

1. Pipette each 50 μ l prepared Standards 1 to 6, Controls and Samples into the respective wells of the coated microtiter strips.
2. Cover the plate with adhesive foil and incubate for 60 minutes at room temperature (20 – 25 °C) on an orbital shaker with medium frequency.
3. Discard or aspirate the contents of the wells, add each 300 μ l 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 μ l enzyme conjugate into all wells.
5. Incubate for 20 minutes at room temperature on an orbital shaker with medium frequency.
6. Washing: Repeat step 3.
7. Pipette each 100 μ l Substrate into all wells.
8. Incubate for 15 to 25 minutes at room temperature (20 - 25 °C) on an orbital shaker with medium frequency.
9. Pipette each 100 μ l 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.

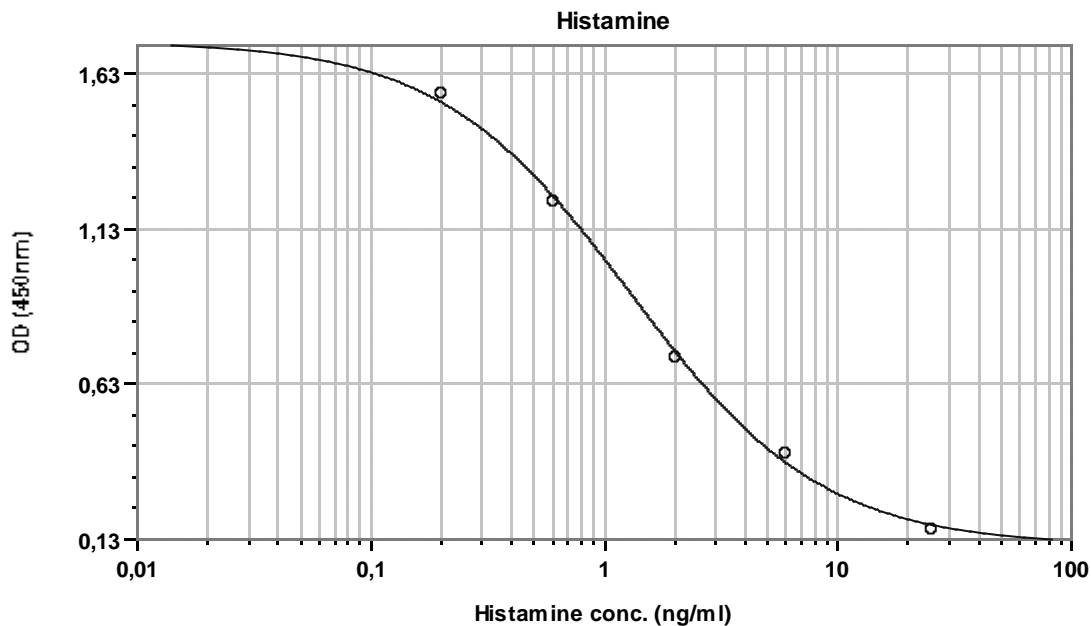
8. Calculation of the Results

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/OD_{max} , and then plotted on the y-axis.

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 urine samples have to be multiplied by a factor of 15.

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

Typical standard curve:



$$y = \left(\frac{A - D}{1 + (x/C)^B} \right) + D$$

	A	B	C	D	R ²
○ Std (Standards: Concentration vs MeanValue)	1,733	1,064	1,287	0,111	0,999

9. Assay Characteristics

9.1 Normal Range

	Reference Range
Plasma	< 1 ng/ml
Urin	< 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.

9.2 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
Plasma	0.06 ng/ml
Urin	0.9 ng/ml

9.3. Specificity (Cross Reactivity)

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

Substanz	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-histidine	< 0.0001

9.4. 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 (%)
Plasma	0.6 – 13.4	101	93 - 111
Urine	6.1 – 140.6	98	94 - 103
Cell Culture Media	1.0 – 12.9	104	91 - 121

9.5. 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 (%)
Plasma	0.5 – 10.0	1 : 20 Equalizing Reagent	106	96 - 111
Urine	7 – 142	1 : 20 dist. water	96	81 - 102
Cell Culture Media	1.1 – 10.3	1 : 10 dist. water	106	99 - 111

9.6. Reproducibility

The reproducibility of the ELISA method was investigated by measuring the intra- and inter-assay-coefficients of variation (cv).

Concentrations in ng/ml

	Range (ng/ml)	Intra-Assay-cv (%)	Range (ng/ml)	Inter-Assay-cv (%)
Plasma	1.2 – 8.7	6.1 – 6.5	1.1 – 3.3	6.2 – 7.3
Urin	24.1 – 89.6	6.6 – 5.7	15.7 – 43.7	7.2 – 11.3
Zellkultur	1.5 – 5.1	6.3 – 8.6	1.3 – 4.1	10.5 – 6.5

10. Literature

- Nettis, E.; Colanardi, A.; Ferrannini, A. (2005):
Antihistamines as Important Tools for Regulating Inflammation
Curr. Med. Chem. – Anti-Inflammatory & Anti-Allergy Agents, 4, 81-89
- Matsumoto, J.; Matsuda, H. (2002):
Mast-cell-dependent histamine release after praziquantel treatment of *Schistosoma japonicum* infection: implications for chemotherapy-related adverse effects
Parasitol Res 88: 888–893
- Belic, A.; Grabnar, I.; Karba, R.; et al. (1999):
Interdependence of histamine and methylhistamine kinetics: modelling and simulation approach
Computers in Biology and Medicine 29, 361-375
- Martens-Lobenhoffer, J.; Neumann, H. (1999):
Determination of 1-methylhistamine and 1-methylimidazoleacetic acid in human urine as a tool for the diagnosis of mastocytosis
Journal of Chromatography B, 721, 135–140
- Prell, G.; Green, J.; Elkashef, A. (1996):
The relationship between urine excretion and biogenic amines and their metabolites in cerebrospinal fluid of schizophrenic patients
Schizophrenia Research 19, 171-176
- Eberlein-König, B.; Ullmann, S.; Thomas, P.; et al. (1995):
Tryptase and histamine release due to a sting challenge in bee venom allergic patients treated successfully or unsuccessfully with hyposensitization
Clinical and Experimental Allergy, Volume 25, pages 704-712
- Koller, D.; Rosenkranz, A.; Pirker, C.; et al. (1992):
Assessment of histamine release from basophils in whole blood by benzylpenicilloyl poly-L-lysine in penicillin-sensitized patients
Allergy: 47: 459-462.
- Marquardt, D.; Wasserman, S. (1982):
Mast Cells in Allergic Diseases and Mastocytosis
West J Med; 137:195-212
- Butchers, P.; Vardey, C.; Skidmore, I.; et al. (1980):
Histamine-Containing Cells from Bronchial Lavage of Macaque Monkeys. Time Course and Inhibition of Anaphylactic Histamine Release
Int. Archs Allergy appl. Immun. 62: 205-212

Pipetting Scheme Sample Preparation

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

shake for 10 seconds

Acyl. Reagent	µl	10	10	10	10	10
---------------	----	----	----	----	----	----

Immediately shake 15 minutes at room temperature, leave plate open

Antiserum	µl	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

cover the plate with adhesive foil
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