DIAGNOSTIKA GMBH

Instruction for use

Histamine ELISA

Enzyme Immunoassay
for the Quantitative Determination of Histamine
in Plasma, Urine, Heparin Whole Blood (Total Histamine)
and Cell Culture Media

IVD CE

REF EA213/96

12 x 8

2 – 8 °C

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Contents

Symbols

I V D	In Vitro Diagnostic Medical Device	CE	EC Declaration of Conformity
CONT	Contents		Expiry Date
LOT	Lot Number	+2 +8 °C	Store
	Manufactured by	Σ	Sufficient for
REF	Catalogue Number		Consult Instructions

Hazard Pictograms

Danger

Warning

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 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.

The Histamine ELISA contains all reagents for the quantitative determination of derivatized histamine in human EDTA plasma, heparin plasma, heparin whole blood (total histamine) and cell culture samples. Derivatization is performed during sample preparation, by quantitatively converting histamine into N-acylhistamine.

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 diagnostic 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.
Bring reagents to room temperature before use and return to 2-8 °C immediately after use.

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** **ACYL-BUFF** 1 vial
6 ml, colour coded blue, ready for use

4.5 **Acylation Reagent** **ACYL-REAG** 3 vials
lyophilised, dissolve content
in 1.5 ml Solvent

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

 Warning  Danger

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

4.8 **Enzyme Conjugate** **CONJ** 1 vial
12 ml, ready for use
goat anti-rabbit-IgG-peroxidase
 Warning

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

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

4.11 **Stop Solution** **STOP** 1 vial
12 ml, ready for use
contains 0.3 M sulphuric acid

4.12 **Reaction Plate** **ACYL-PLATE** 2 plates
for acylation

4.13 **Equalizing Reagent** **EQUA-REAG** 1 vial
lyophilzed, dissolve contents with distilled water,
volume: see vial label

4.14 **Releasebuffer** **RELEASE BUFF** 2 vials
21 ml, ready to use

Additional materials and equipment required but not provided:

Pipettes for 50 and 100 µl

- Multipette
- Orbital shaker
- Multichannel pipette or Microplate washing device
- Microplate photometer (450 nm)
- Distilled water
- Centrifuge
- Heating block or water bath
- 1.5 ml reaction tubes

5. Specimen 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.

Heparin whole blood (Total Histamine)
Collect whole blood in a blood vial containing heparin as anticoagulant (e.g. LH-Monvette™) and mix carefully.

The samples can be stored up to 24 hours at 20 - 25 °C. Do not store at 2 - 8 °C, as this will lead to agglutination of leukocytes. Avoid exposure to direct sun light.

Bring release buffer to room temperature. Determinations in duplicate are recommended.

1. Pipette 20 µl of heparin whole blood into a reaction tube.
2. Add 480 µl release buffer, close tube and mix.
3. Incubate in heating block or water bath for 10 min at 90 °C.
4. Incubate in water bath (room temperature) for 10 min.
5. Centrifuge for 10 min at 2,000g and room temperature.
6. Use 50 µl of supernatant for sample preparation (acylation) according to section 6.2.

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 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.

6.2. Preparation of Samples (Acylation)

Bring reagents and samples to room temperature. Determinations in duplicates are recommended. As wells of the reaction plate (Acyl-Plate) can be used only once, it is recommend to mark the respective wells.

1. Pipette 50 µl standard 1 - 6, 50 µl control 1 & 2 and 50 µl EDTA plasma, 20 µl heparin plasma, 50 µl urine sample (diluted 1:15 with dist. water, see section 5), 50 µl of heparin whole blood (heated 1:25 in release buffer, see section 5) or 50 µl cell culture media samples into the respective wells of the reaction plate.

2. Pipette 50 µl Acylation Buffer into each well.

3. Pipette 50 µl dist. water in each well containing heparin plasma and EDTA plasma samples.

4. Pipette 50 µl Equalizing Reagent into each well containing standards, controls, urine, heparin whole blood and cell culture media samples. Pipette 30 µl Equalizing Reagent into each well containing heparin plasma. Do not pipette Equalizing Reagent into wells containing EDTA plasma samples. Mix the reaction plate for 10 seconds.

5. Pipette 10 µl Acylation Reagent into each well 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. Further, solvent is volatile and the dissolved Acylation Reagent evaporates quickly. Therefore, please do not use a tray together with a multichannel pipette for pipetting Acylation Reagent. Rather, use an Eppendorf multipette (or similar device), and fill it directly from the vial of dissolved Acylation Reagent and pipette 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 each well.

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 50 µl each for the ELISA.

7. Test Procedure ELISA

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

1. Pipette 50 µl of acylated 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.

3. Discard or aspirate the contents of the wells, add 300 µl Wash Buffer per well, 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 100 µl enzyme conjugate into each well.

5. Incubate for 20 minutes at room temperature on an orbital shaker with medium frequency.

6. Washing: Repeat step 3.

7. Pipette 100 µl Substrate into each well.

8. Shake for 10 seconds on an orbital shaker.
   Incubate for 20 ± 5 minutes at room temperature (20 - 25 °C) on the table without shaking. Cover with a large box.

9. Pipette 100 µl Stop Solution into each well.

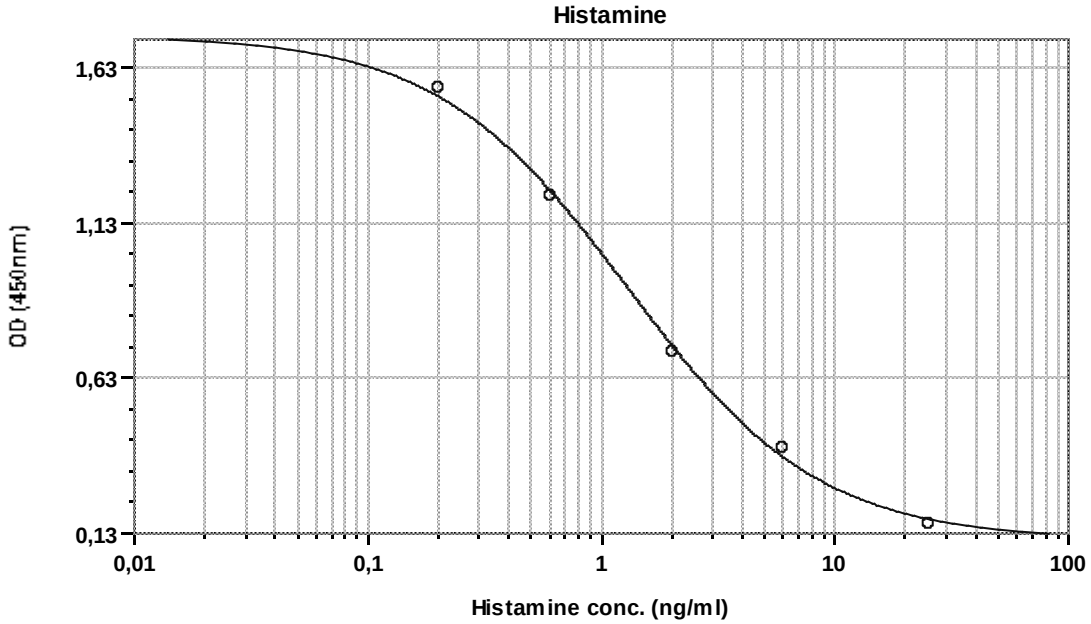
10. Read the optical density at 450 nm (reference wavelength between 570 and 650 nm) in a microplate photometer within 15 minutes.

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. 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.
The read concentration of the heparin whole blood samples has to be multiplied by a factor of 25.
Conversion: 1 ng/ml corresponds to 9,0 nmol/l

Typical standard curve:



	y = ((A - D)/(1 + (x/C)^B)) + D:	A	B	C	D	R^2
○	Std (Standards: Concentration vs MeanValue)	1,733	1,064	1,287	0,111	0,999

Quality Control: The results are only valid, when the kit controls are within the range indicated on the QC certificate. Otherwise, the test must be repeated.

9. Assay Characteristics

9.1 Normal Range

	Reference Range
EDTA Plasma	< 1 ng/ml
Heparin Plasma	< 4.5 ng/ml
Heparin whole blood	10 - 100 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

	Sensitivity	Calculation
EDTA Plasma, cell culture sample	0.06 ng/ml	OD_{Cal1} - 2xSD
Heparin Plasma	0.15 ng/ml	OD_{Cal1} - 2xSD
Heparin whole blood	1.5 ng/ml	OD_{Cal1} - 2xSD
Urine	0.9 ng/ml	OD_{Cal1} - 2xSD

9.3. Specificity (Cross Reactivity)

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-histidine	< 0.0001

9.4. Recovery after spiking

	Range (ng/ml)	Mean (%)	Range (%)
EDTA Plasma	0.6 – 13.4	101	93 - 111
Heparin Plasma	0.8 – 36.0	104	87 - 112
Heparin whole blood	40 – 145	101	96 - 106
Urine	6.1 – 140.6	98	94 - 103
Cell Culture Media	1.0 – 12.9	104	91 - 121

9.5. Linearity

	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
H. whole blood	22 – 148	1 : 7 dist. water	103	96 - 106
Urine	7 – 142	1 : 20 dist. water	96	81 - 102
Cell Culture Medium	1.1 – 10.3	1 : 10 dist. water	106	99 - 111

9.6. Reproducibility

	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
H. whole blood	36 – 102	9.3 – 6.8 %	36.4 – 128	11.2 – 6.3 %
Urine	24.1 – 89.6	6.6 – 5.7	15.7 – 43.7	7.2 – 11.3
Cell Culture Medium	1.5 – 5.1	6.3 – 8.6	1.3 – 4.1	10.5 – 6.5

9.6. Comparision of methods

Matrix	Method	Correlation
EDTA-Plasma	LC/MS	Y = 1.22 x LC/MS - 0.33; R = 0.950; N = 24
EDTA-Plasma	Elisa A	Y = 0.96 x ELISA A + 0.19; R = 0.893; N = 43
EDTA-Plasma	Elisa B	Y = 1.22 x ELISA B - 0.89; R = 0.936; N = 41
EDTA-Plasma	Elisa C	Y = 0.65 x ELISA C - 0.01; R = 0.920; N = 45
Heparin-Plasma	LC/MS	Y = 1.03 x LC/MS - 0.22; R = 0.987; N = 16
H. whole blood	LC/MS	Y = 1.13 x LC/MS + 0.65; R = 0.993; N = 10
Urine	LC/MS	Y = 0.98 x LC/MS + 1.7; R = 0.978; N = 32
Urine	Elisa A	Y = 1.06 x ELISA A - 2.2; R = 0.965; N = 56
Urine	Elisa B	Y = 1.16 x ELISA B - 2.1 R = 0.936; N = 22
Urine	Elisa C	Y = 0.88 x ELISA C + 6.7; R = 0.944; N = 20

10. Literature

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Pipetting Scheme Sample Preparation

(µl)	Standards	Controls	EDTA Plasma	Heparin Plasma	Urine	H. whole blood	Cell Culture Medium
Standard 1 - 6	50						
Control 1 & 2		50					
EDTA Plasma			50				
Heparin Plasma				20			
Urine (dil. 1:15*)					50		
H. whole blood (dil. 1:25*)						50	
Cell Culture Medium							50
Acyl. Buffer	50	50	50	50	50	50	50
Dist. Water			50	50			
Equalizing Reag.	50	50		30	50	50	50

* see section 5

shake for 10 seconds

Acyl. Reagent	10	10	10	10	10	10	10

Immediately shake for 15 minutes at room temperature, do not cover

Antiserum	50	50	50	50	50	50	50

shake for 30 minutes at room temperature, do not cover

Pipetting Scheme ELISA

(µl)	Standards	Controls	Sample
Acyl. Standard 1 - 6	50		
Acyl. Control 1 & 2		50	
Acyl. Sample			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 10 seconds
incubate for 15 - 25 minutes at room temperature on table, cover with large box

Stop Solution µl	100	100	100

Reading of absorbance at 450 nm