DIAGNOSTIKA GMBH

Instructions for Use

Histamine ELISA

Enzyme Immunoassay for the
Quantitative Determination of
Histamine

in human Heparin Plasma, EDTA-Plasma, Urine, Heparin Whole Blood (Total Histamine) and Stool

CE IVD

Item No. EA213/96

12 x 8

2 – 8 °C

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Table of Contents

Symbols

IVD	In Vitro Diagnostic Medical Device	CE	EC Declaration of conformity
CONT	Content		Expiry Date
LOT	Lot Number		Store at
	Manufactured by		Sufficient for ... determinations
REF	Catalogue Number manufacturer		Consult Instructions for Use

The symbols of the components of the kit are described in section 4 Contents of the Kit.

Changes to declare

Version _12: Research Use Only instructions for cell culture samples removed (available in separate IFU). Further additions/changes are highlighted in gray.

1 Introduction and Principle of the Test

Histamine belongs to the group of biogenic amines and is formed from the amino acid histidine. Histamine triggers physiological and pathophysiological reactions in the human organism. It is involved in the defense against substances seen as foreign to the body and its pathological reactions lead to symptoms such as allergy and asthma.

The Histamine ELISA Kit contains reagents for the quantitative determination of derivatized histamine in human urine, EDTA-plasma and heparin plasma, heparin whole blood (total histamine) and stool. Derivatization takes place during sample preparation. In this process, histamine is quantitatively converted into N-acylhistamine by the acylating reagent.

The Histamine ELISA is a competitive enzyme immunoassay. Antigen bound to the solid phase and free antigen in solution compete for a defined number of antibody binding sites. When the system is in equilibrium, the unbound antigen-antibody complex is removed in a washing step and the correspondingly bound complex is detected using an anti-rabbit-IgG-peroxidase conjugate and determined via the conversion of tetramethylbenzidine (TMB). The TMB/POD reaction is stopped and the absorbance measured at 450 nm. The concentration of the antigen-antibody complex bound to the solid phase is inversely proportional to the concentration of the antigen in the sample.

2 Precautions

- For in vitro diagnostic use only. For professional use only.
- Before carrying out the test, the valid instructions for use, as included in this kit, should be read completely and the content understood.
- Material of human origin used in the preparation of this kit has been tested and found non-reactive for HBsAg, HCV or HIV I/II antibodies, but should non-the-less, be handled as potentially infectious.
- 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.
- Individual components of different lots and test kits should not be interchanged. The expiry dates and storage conditions stated on the packaging and the labels of the individual components must be observed.
- When handling the reagents, controls and patient samples, the current laboratory safety guidelines and good laboratory practice should be observed.
- Wear protective clothing, disposable gloves, and safety goggles while performing the test.
- Some components of this kit contain hazardous reagents. These components are marked with the adequate hazard label. Further information is in section 4 and in the corresponding MSDS.
- Avoid any actions that could result in ingestion, inhalation or injection of the reagents. Never pipette by mouth.
- Avoid contact with individual reagents.
- Dispose of waste according to state and local environmental protection regulations.
- The quality control guidelines in the medical laboratory regarding the inclusion of control samples and/or pooled samples should be observed.
- Some components contain small amounts of sodium azide as a preservative. Prevent the formation of heavy metal azides in the drain system by flushing with copious amounts of water.

3 Storage and Stability

The kit is shipped at ambient temperature. Upon arrival, store the kit at 2 – 8 °C to keep it stable until its expiry date. Once opened the kit is stable until its expiry date. The shelf life of the ready-to-use reagents is indicated on the respective bottle label. For stability of prepared reagents refer to 6.1..

Reagents must equilibrate to room temperature before use and refrigerated immediately after use.

4 Contents of the Kit

MT-Strips 8 wells each, break apart, precoated with N-acyl-histamine	**STRIPS**	12 strips
Standards 1 – 6 each 4 ml, ready for use	**CAL 1 - 6**	6 vials
Control 1 & 2 Each 4 ml, ready for use Range: see QC certificate	**CON 1 & 2**	2 vials
Acylation Reagent 3 ml lyoph., dissolve content in SOLVENT	**ACYL-REAG**	3 vials
Acylation Buffer 14 ml, ready for use, color coded blue	**ACYL-BUFF**	1 vial
Solvent 11 ml, ready for use, color coded yellow	**SOLVENT**	1 vial
Antiserum 6 ml, ready for use, color coded yellow, rabbit-anti-N-acyl-histamine	**AS**  Warning	1 vial
Enzyme Conjugate 13 ml, ready for use, anti-rabbit-IgG-peroxidase	**CONJ**  Warning	1 vial
Wash Buffer 20 ml, 50 x concentrated	**WASH**	1 vial

Substrate 13 ml, TMB solution, ready for use	**SUB**	1 vial
Stop Solution 13 ml, ready for use, contains 0.3 M sulphuric acid	**STOP**	1 vial
Acylation Plate For sample preparation (acylation)	**ACYL-PLATE**	2 plates
Equalizing Reagent lyoph., dissolve contents with 6 ml ACYL-BUFF	**EQUA-REAG**	1 vial
Releasebuffer 21 ml, ready for use	**RELEASE-BUFF**	2 vials
Adhesive Foil ready for use	**FOIL**	2 pieces

Additional materials and equipment required but not provided:

- Pipettes for 10, 20, 50, 100 and 500 µl
- Multipette
- Orbital shaker
- Vortex mixer and roll mixer
- Multichannel pipette or Microplate washing device
- Microplate photometer (450 nm)
- Distilled water
- Centrifuge
- Paper towels, pipette tips, timer
- Heating block or water bath
- 1.5 ml reaction tubes
- Stool collection tubes (see 5.4)

5 Specimen Collection and Storage

Repeated freezing and thawing of samples should be avoided.

5.1 Plasma

EDTA-plasma or heparin plasma can be used. However, as Histamine concentrations have been found to be lower in EDTA-plasma, it is recommended to use heparin plasma. Haemolytic, lipaemic and icteric samples should not be used.

The samples can be stored up to 6 hours at 2 – 8 °C. For longer storage (up to 6 months) the samples must be frozen at -20 °C.

5.2 Urine

Spontaneous urine can be used for this test as well as collected urine. In the case of collected urine, 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 (please, take caution) as preservative. During collection, store in the dark. Determine the total volume and take an aliquot for the measurement. For patients with suspected kidney disorders the creatinine concentration should be determined in addition. Urine samples can be stored at -20 °C for at least 6 months.

Before use, mix urine and centrifuge.

5.3 Total Histamine

To determine total histamine, heparin whole blood must be used. 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:

- 3 days at 20 – 25 °C. Do not store at 2 - 8 °C. Avoid exposure to direct sun light.
- One week at -20 °C.

Equilibrate RELEASE BUFF to room temperature. Determinations in duplicate are recommended.

1. Pipette **20 µl** of heparin whole blood into a reaction tube.
2. Add **500 µl** release buffer RELEASE BUFF, 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 2,000 x g at room temperature.
6. Use 50 µl of supernatant for sample preparation (acylation) according to section 6.2.

5.4 Stool

Stool collection tubes are required for specimen collection and must be used according to the instructions included. Stool collection tubes must be ordered separately.

Procedure with already filled stool collection tube:

Before use in this assay, invert stool collection tube several times or vortex. Then allow tube to stand upright and contents to settle for 5 minutes or centrifuge for 5 min at 2,000 x g at room temperature. Remove adhesive tape and twist off blue sleeve to pipette 50 µl of the supernatant for sample preparation (acylation, see 6.2).

Procedure after taking your own sample, immediately before use in this assay: Vortex filled stool collection tube at maximum speed until there is no more stool in the notches of the sampling stick. Tilting the stool collection tube while vortexing can be helpful. Then allow the stool collection tube to stand for 20 minutes for complete extraction. Invert or vortex the stool collection tubes. Allow tubes to stand upright and contents to settle for 5 minutes or centrifuge for 5 min at 2,000 x g at room temperature. Remove adhesive tape and twist off blue sleeve to pipette 50 µl of the supernatant for sample preparation (acylation, see 6.2).

The extracted samples can be stored at room temperature for up to 2 days. For longer storage, the extracted samples must be frozen at ≤ -20 °C.

6 Preparation of Reagents and Samples

6.1 Preparation of Reagents

Equilibrate reagents to room temperature before use.

6.1.1 Equalizing Reagent

Dissolve the contents of EQUA-REAG by pipetting 6 ml of ACYL-BUFF into the vial. Mix shortly on a vortex mixer and leave on a roll mixer for at least 20 minutes until dissolved completely. Thereby, avoid excessive formation of foam. The reconstituted Equalizing Reagent should be stored at -20 °C and is stable until expiry date printed on vial label.

6.1.2 Wash Buffer

Dilute the contents of the 20 ml concentrated (50x) wash buffer WASH with dist. water to a total volume of 1000 ml, mix briefly.

The diluted wash buffer can be stored at 2 – 8 °C for a maximum period of 4 weeks. For longer storage freeze at -20 °C.

Should the kit be used in several runs, it is recommended to prepare only the required amount of wash buffer for each run.

6.1.3 Acylation Reagent

Remove the required amount of vials of acylation reagent ACYL-REAG from the foil pouch, leave the remaining vials in the foil pouch together with the desiccant and close it carefully.

Dissolve the content of one bottle in 3 ml SOLVENT and shake for 5 minutes on a roll mixer or similar mixer. The Acylation Reagent must always be prepared immediately before performing the assay and is stable for approximately 3 hours. After use discard leftovers of the acylation reagent.

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 two vials of Acylation Reagent. Discard after use.

All other reagents are ready for use.

6.2 Preparation of Samples (Acylation)

Equilibrate 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 **CAL 1 - 6**
   50 µl control 1 & 2 **CON 1 & 2**
   50 µl of extracted heparin whole blood (diluted 1:26 in release buffer and heated, see 5.3)
   50 µl extracted stool sample
   10 µl urine sample
   50 µl EDTA-plasma
   50 µl heparin plasma
   into the respective wells of the reaction plate ACYL-PLATE.
2. Pipette
   50 µl of dist. water into each well containing **urine**.
   50 µl dissolved Equalizing Reagent EQUA-REAG (see 6.1.1) into each well containing **standards, controls and heparin whole blood, stool or urine**. Do not add Equalizing Reagent to wells with EDTA-plasma or heparin plasma.
   50 µl Acylation Buffer ACYL-BUFF into each well containing **EDTA-plasma or heparin plasma**.
3. Shake for 10 seconds on a horizontal shaker.
4. Pipette **50 µl dissolved Acylation Reagen**t ACYL-REAG (see 6.1.3) into each well and proceed with the next step, immediately.
5. Incubate for 20 minutes at room temperature on an orbital shaker at medium frequency.
6. Pipette **50 µl Antiserum** AS into each well.
7. Incubate for 30 minutes at room temperature on an orbital shaker at medium frequency.

   Take 50 µl each for the ELISA.

7 Test Procedure ELISA

1. Transfer **50 µl** each of **acylated Standards 1 to 6, Controls 1 & 2** and **Samples** from the Acylation Plate into the respective wells of the coated microtiter strips [STRIPS]. Leave strips, which are not needed, in the bag with the desiccant and close it thoroughly.
2. Seal strips with adhesive foil [FOIL].
   Incubate for 60 minutes at room temperature (20 – 25 °C) on an orbital shaker at medium frequency.
3. Discard or aspirate the contents of the wells, pipette **300 µl diluted Wash Buffer** [WASH] (s. 6.1.2) into each well and again discard or aspirate the contents of the wells. Remove residual liquid by tapping the inverted plate on clean absorbent paper towel.
   Repeat the washing procedure 3 times.
   Alternatively, a washing device may be used.
4. Pipette **100 µl Enzyme Conjugate** [CONJ] into each well.
5. Incubate for 20 minutes at room temperature on an orbital shaker at medium frequency.
6. Washing: Repeat step 3.
7. Pipette **100 µl Substrate** [SUB] 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** [STOP] into each well.
   Shake for 10 seconds on an orbital shaker.
10. Read the optical density at 450 nm (reference wavelength between 570 nm and 650 nm) in a microplate photometer within 15 minutes.

8 Calculation of the Results

Standard	1	2	3	4	5	6
ng/ml	0	0.3	1	3	10	30
nmol/l	0	2.7	9.0	27	90	270

Conversion: Histamine: 1 ng/ml corresponds to 9.0 nmol/l

The concentration of the standards (x-axis, logarithmic) are plotted against their corresponding optical density (y-axis, linear). When using analysing software, it is recommended to use the Four Parameter Logistic (4PL) Regression (alternatively: cubic-spline or logit-log).

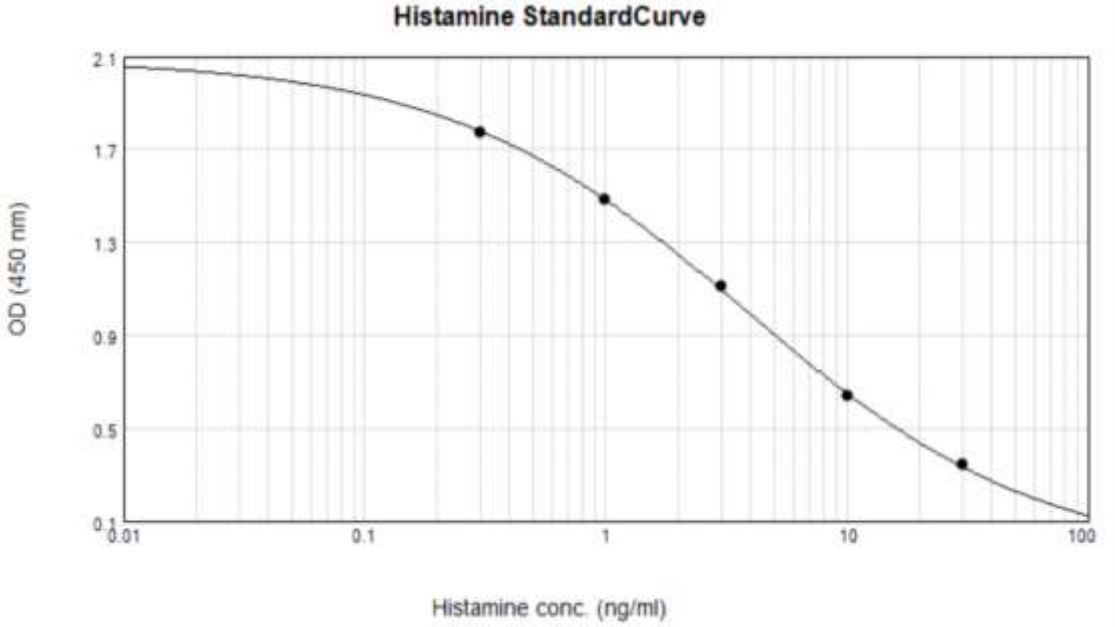
The concentration of the controls, EDTA-plasma and heparin plasma samples in ng/ml can be read directly from the standard curve.

The read concentration of urine samples in ng/ml must be multiplied by a factor of 5.

The read concentration of the heparin whole blood samples in ng/ml must be multiplied by a factor of 26.

The read concentration of the stool samples must be multiplied by a factor of 150, which accounts for the amount of stool used in the assay. The result is expressed as ng/g.

Typical standard curve (example, do not use for calculation)**:**



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

Matrix	Reference Range
EDTA Plasma	< 1 ng/ml
Heparin Plasma	< 4.5 ng/ml
Heparin whole blood	10 - 100 ng/ml
Urine	< 45 µg/day
Stool	< 600 ng/g

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

9.2 Analytical Sensitivity

Matrix	Lower Detection Limit	Calculation
EDTA Plasma, Heparin Plasma	0.09 ng/ml	OD_{Cal1} - 2xSD
Heparin whole blood	2.3 ng/ml	OD_{Cal1} - 2xSD
Urine	0.45 ng/ml	OD_{Cal1} - 2xSD
Stool	13.4 ng/ml	OD_{Cal1} - 2xSD

9.3 Analytical Specificity (Cross Reactivity)

Substance	Cross Reactivity (%)
Histamine	100
1-methyl histamine	< 0.05
3-methyl histamine	0.1
1-methyl-4-imidazol-acetic acid	< 0.0002
Imidazol-4-acetic acid	< 0.0005
L-histidine	< 0.0002

9.4 Recovery after spiking

Matrix	Range (ng/ml)	Mean (%)	Range (%)
EDTA Plasma	0.78 – 12.6	98	96 - 102
Heparin Plasma	1.8 – 13.0	105	94 - 111
Heparin whole blood	57 – 157	104	99 - 111
Urine	14 – 109	101	93 - 107
Stool	206 – 1773	103	92 - 109

9.5 Linearity

Matrix	Range(ng/ml)	Highest Dilution	Mean (%)	Range (%)
EDTA-Plasma	3.1 – 14.2	1 : 5 Equalizing Reagent	107	102 - 110
Heparin Plasma	3.2 – 16.0	1 : 5 Equalizing Reagent	96	89 - 101
H. whole blood	27 – 389	1 : 15 dist. water	100	96 - 103
Urine	6.8 - 78	1 : 10 dist. water	94	87 - 102
Stool	210 – 2052	1 : 10 dist. water	97	88 - 102

9.6 Reproducibility

Matrix	Range (ng/ml)	Intra-Assay-cv (%)
EDTA-Plasma	1.7 – 9.1	10.5 – 7.0 %
Heparin Plasma	1.6 – 9.9	11.1 – 5.5 %
H. whole blood	54 – 158	9.8 – 8.4 %
Urine	13 – 64	8.9 – 9.4 %
Stool	179 – 1096	10.6 – 8.7 %

9.7 Comparison of methods

Matrix	Method	Correlation
EDTA-Plasma	LC/MS	Y = 0.87 x LC/MS - 0.06; R = 0.979; N = 13
Heparin Plasma	LC/MS	Y = 1.01 x LC/MS + 0.089; R = 0.973; N = 28
H. whole blood	Elisa A	Y = 1.11 x ELISA A - 4.71; R = 0.994; N = 21
Urine	LC/MS	Y = 1.05 x LC/MS + 3.2; R = 0.964; N = 32
Stool	Elisa A	Y = 0.87 x ELISA A + 63; R = 0.961; N = 51

9.8 Calibration

The calibration is carried out by weighing the pure substance. The correctness of the method was determined by comparing the methods (9.7).

9.9 Limitation of Method

The result of the Histamine-Elisa is to be seen in connection with other diagnostic procedures and the anamnesis and the resulting questions.

Samples measured above the highest standard must be diluted with the appropriate medium (see 9.5) and determined again. The values of diluted samples must be multiplied by the appropriate dilution factor.

9.10 Interferences

Hemolytic, lipemic and icteric specimens should not be used. Do not use non-acidified urine collection. Renal dysfunction may influence histamine determination.

10 Literature

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Pipetting Scheme - Sample Preparation

		Standards	Controls	EDTA- / Heparin Plasma	Stool	Urine	H. whole blood
ACYL-PLATE:							
CAL 1 - 6	µl	50					
CON 1 & 2	µl		50				
EDTA-plasma / Heparin Plasma	µl			50			
Stool					50		
Urine	µl					10	
H. whole blood (dil. 1:26)	µl						50
Dist. Water	µl					50	
EQUA-REAG	µl	50	50		50	50	50
ACYL-BUFF	µl			50			

Shake for 10 seconds

ACYL-REAG	µl	50	50	50	50	50	50

Immediately, shake for 20 minutes at room temperature

AS	µl	50	50	50	50	50	50

Shake for 30 minutes at room temperature

Pipetting Scheme – ELISA

		Acyl. Standards	Acyl. Controls	Acyl. Samples
STRIPS:				
Transfer from ACYL-PLATE to STRIPS	µl	50	50	50

Cover with FOIL
Shake for 1 hour at room temperature
4 x washing

CONJ	µl	100	100	100

Shake for 20 minutes at room temperature
4 x washing

SUB	µl	100	100	100

Shake for 10 seconds
Incubate for 20 ± 5 minutes at room temperature, covered with large box, no shaking

STOP	µl	100	100	100

Shake for 10 seconds
Determine absorbance at 450 nm (ref 570 – 650 nm)