Instructions for Use

Histamine ELISA

Enzyme immunoassay for the quantitative determination of histamine in human plasma and urine. Further the test can be used for research of cell culture supernatants.

REF **40-371-25010**

Σ **96**

 **2-8°C**

U.S.: *For research use only.*
Not for use in diagnostic procedures.

1. INTENDED USE

Enzyme immunoassay for the quantitative determination of histamine in human plasma and urine. Further the test can be used for research of cell culture supernatants.

2. SUMMARY AND EXPLANATION

In humans, histamine (ß-imidazolethylamine) is the most important mediator and is mostly found in the initial phase of an anaphylactic reaction ("immediate type" allergy). Histamine is derived by the enzymatic decarboxylation of histidine. In the organism, histamine is present in nearly all tissues, and it is mainly stored in the metachromatic granula of mast cells and the basophilic leukocytes. It is present in an inactive bound form and is only released as required. Like several other mediators, histamine does not only mediate various clinical symptoms of anaphylaxis but also induces a series of effects which are directed towards a termination of the anaphylactic reaction. The biological action of histamine in tissue is guaranteed by three different surface receptors, i.e. H1, H2 and H3 receptors. Of clinical interest in the histamine determination is the quantification of the histamine release from basophilic leukocytes in allergies of the "immediate type" as well as of the histamine quantity which is present in various body fluids (plasma, urine, cell culture supernatants), after allergen administration.

3. TEST PRINCIPLE

Solid phase enzyme-linked immunosorbent assay (ELISA) based on the competition principle. An unknown amount of antigen present in the sample and a fixed amount of enzyme labelled antigen compete for the binding sites of the antibodies coated onto the wells. After incubation the wells are washed to stop the competition reaction. After the substrate reaction the intensity of the developed color is inversely proportional to the amount of the antigen in the sample. Results of samples can be determined directly using the standard curve.

4. WARNINGS AND PRECAUTIONS

1. For research use only. For professional use only.
2. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
3. In case of severe damage of the kit package please contact GenWay in written form, latest one week after receiving the kit. Do not use damaged components in test runs, but keep safe for complaint related issues.
4. Obey lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.
5. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.
6. Reagents of this kit containing hazardous material may cause eye and skin irritations. See MATERIALS SUPPLIED and labels for details.
7. Chemicals and prepared or used reagents have to be treated as hazardous waste according to national biohazard and safety guidelines or regulations.
8. Avoid contact with Stop solution. It may cause skin irritations and burns.
9. Some reagents contain sodium azide (NaN_3) as preservatives. In case of contact with eyes or skin, flush immediately with water. NaN_3 may react with lead and copper plumbing to form explosive metal azides. When disposing reagents, flush with a large volume of water to avoid azide build-up.
10. All reagents of this kit containing human serum or plasma have been tested and were found negative for HIV I/II, HBsAg and HCV. However, a presence of these or other infectious agents cannot be excluded absolutely and therefore reagents should be treated as potential biohazards in use and for disposal.

5. STORAGE AND STABILITY

The kit is shipped at ambient temperature and should be stored at 2-8°C. Keep away from heat or direct sun light. The storage and stability of specimen and prepared reagents is stated in the corresponding chapters.

The microtiter strips are stable up to the expiry date of the kit in the broken, but tightly closed bag when stored at 2–8°C.

6. SPECIMEN COLLECTION AND STORAGE

Plasma (EDTA, Heparin)

The usual precautions for venipuncture should be observed. It is important to preserve the chemical integrity of a blood specimen from the moment it is collected until it is assayed. Do not use grossly hemolytic, icteric or grossly lipemic specimens. Samples appearing turbid should be centrifuged before testing to remove any particulate material.

Storage:	2-8°C	≤ -20°C (Aliquots)	≤ -70°C (Aliquots)	Keep away from heat or direct sun light. Avoid repeated freeze-thaw cycles. Ship samples frozen.
Stability:	5 h	3 mon	2 y	

Urine

It is possible to use spontaneous as well as 24 h urine. The total volume of urine excreted during a 24 h period should be collected and mixed in a single bottle containing 10 - 15 mL of 6 N HCl as preservative. Determine total volume for calculation of results. **Mix and centrifuge samples before use in the assay.**

	spontaneous	acidified		
Storage:	2-8°C	2-8°C	≤ -20°C (Aliquots)	Keep away from heat or direct sun light. Avoid repeated freeze-thaw cycles. Ship samples frozen.
Stability:	8 h	3 d	6 mon	

Cell Culture Supernatants

Cell culture supernatants may be used without special precautions.

Cell culture media may contain histamine in higher amounts.

Whole Blood

The histamine release is performed with heparinized whole blood. For further information see instructions for use of Histamine Release (REF RE95000).

7. MATERIALS SUPPLIED

⚠ The reagents provided with this kit are sufficient for single determinations in the sample preparation and duplicates in the assay. Additional reagents are available upon request.

Quantity	Symbol	Component
1 x 12x8	**MTP**	**Microtiter Plate** Break apart strips. Coated with anti-rabbit antiserum (goat).
1 x 5 mL	**ANTISERUM**	**Histamine Antiserum** Blue colored. Ready to use. Contains Antiserum (rabbit), Tris buffer, 0.01 % Thimerosal.
1 x 75 µL	**ENZCONJ CONC**	**Enzyme Conjugate** Concentrate (200x) Contains Histamine, conjugated to peroxidase.
7 x 0.4 mL	**CAL P A-G LYO**	**Plasma Standard A-G** lyophilized For calibration of plasma samples. Standard A = Diluent for plasma samples. Contains Histamine, human plasma. Exact concentrations see labels or QC Certificate.
2 x 0.4 mL	**CONTROL P 1+2 LYO**	**Plasma Control 1+2** lyophilized Contains Histamine, human plasma. Concentrations / acceptable ranges see QC Certificate.
1 x 2.0 mL 5 x 0.25 mL	**CAL U/C A-F**	**Urine/Cell Culture Standard A-F** 0; 2.7; 8.1; 24.3; 73; 219 ng/mL* Ready to use. For calibration of urine and cell culture samples. Standard A = Diluent for urine samples. Contains Histamine, 0.1 M HCl.

Quantity	Symbol	Component
2 x 0.25 mL	CONTROL U/C 1+2	**Urine Control 1+2** Ready to use. Contains Histamine, human urine (acidified). Concentrations / acceptable ranges see QC Certificate.
1 x 1.5 mL	ACYLREAG	**Acylation Reagent** Ready to use. Contains DMF.
1 x 60 mL	ASSAYBUF CONC	**Assay Buffer** Concentrate (5x) Contains Tris buffer, Tween, BSA, 0.05 % Thimerosal.
1 x 50 mL	WASHBUF CONC	**Wash Buffer** Concentrate (20x) Contains phosphate buffer, Tween, 0.1 % Thimerosal.
1 x 10 mL	INDICATORBUF	**Indicator Buffer** Purple colored. Ready to use. Contains Tris buffer, phenol red (color change at pH < 7.5), 0.01 % Thimerosal.
1 x 0.9 mL	TMB SUBS CONC	**TMB Substrate Solution** Concentrate (31x) Contains TMB, Buffer, stabilizers.
1 x 27 mL	TMB BUF	**TMB Substrate Buffer** **Store protected from light.** Ready to use. Contains H_2O_2, citrate buffer, stabilizers.
1 x 12 mL	TMB STOP	**TMB Stop Solution** Ready to use. 1 M H_2SO_4.
3 x	FOIL	**Adhesive Foil**

8. MATERIALS REQUIRED BUT NOT SUPPLIED

1. Micropipettes (Multipette Eppendorf or similar devices, < 3% CV). Volumes: 10; 20; 50; 100; 1000 µL
2. Disposable glass test tubes (12 x 75 mm)
3. Rack for test tubes
4. Orbital shaker (500 rpm)
5. Vortex mixer
6. 8-Channel Micropipettor with reagent reservoirs
7. Wash bottle, automated or semi-automated microtiter plate washing system
8. Microtiter plate reader capable of reading absorbance at 450 nm (reference wavelength 600-650 nm)
9. Bidistilled or deionised water
10. Paper towels, pipette tips and timer

9. PROCEDURE NOTES

1. Any improper handling of samples or modification of the test procedure may influence the results. The indicated pipetting volumes, incubation times, temperatures and pretreatment steps have to be performed strictly according to the instructions. Use calibrated pipettes and devices only.
2. Once the test has been started, all steps should be completed without interruption. Make sure that required reagents, materials and devices are prepared ready at the appropriate time. Allow all reagents and specimens to reach room temperature (18-25 °C) and gently swirl each vial of liquid reagent and sample before use. Mix reagents without foaming.
3. Avoid contamination of reagents, pipettes and wells/tubes. Use new disposable plastic pipette tips for each reagent, standard or specimen. Do not interchange caps. Always cap not used vials. Do not reuse wells/tubes or reagents.
4. Some components contain ≤ 250 µL solution. Take care that the solution is completely on the bottom of the vial before opening.
5. It is advised to determine samples in duplicate to be able to identify potential pipetting errors.
6. Use a pipetting scheme to verify an appropriate plate layout.
7. Incubation time affects results. All wells should be handled in the same order and time sequences. It is recommended to use an 8-channel Micropipettor for pipetting of solutions in all wells.
8. Microplate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or an automatic microplate washing system. Do not allow the wells to dry between incubations. Do not scratch coated wells during rinsing and aspiration. Rinse and fill all reagents with care. While rinsing, check that all wells are filled precisely with Wash Buffer, and that there are no residues in the wells.

9. Humidity affects the coated wells/tubes. Do not open the pouch until it reaches room temperature. Unused wells/tubes should be returned immediately to the resealed pouch including the desiccant.

10. PRE-TEST SETUP INSTRUCTIONS

⚠	The contents of the kit for 96 determinations can be divided into 3 separate runs. **The volumes stated below are for one run with 4 strips (32 determinations).**

10.1. Preparation of lyophilized or concentrated Components

Dilute/ dissolve	Component		Diluent	Rela-tion	Remarks	Storage	Stability
20 mL	**Assay Buffer**	ad 100 mL	bidist. water	1:5		2-8°C	2 w
15 mL	**Wash Buffer**	ad 300 mL	bidist. water	1:20	Resolve crystals at 18-25°C.	2-8°C	4 w
	Plasma Standards	with 0.4 mL	bidist. water		Let stand for 15 min. Mix without foaming.	-20°C	1 mon
	Plasma Controls	with 0.4 mL	bidist. water		Let stand for 15 min. Mix without foaming.	-20°C	1 mon
10 µL(*)	**Enzyme Conjugate**	with 2 mL	diluted Assay Buffer	1:200	Prepare freshly and use only once.	18-25°C	30 min
300 µL	**TMB Substrate Solution**	with 9 mL	TMB Substrate Buffer	1:31	Prepare freshly and use only once.	18-25°C	10 min

(*) Prior to dilution make sure that no liquid will remain in the stopper.

10.2. Dilution of Samples

Samples suspected to contain concentrations higher than the highest standard have to be diluted accordingly with Standard A of urine or plasma.

10.3. Acylation of Samples

Sample preparation should be performed in glass tubes.

⚠	It is not possible to determine acylated urine or cell culture samples by use of the plasma standard curve or to determine acylated plasma samples by use of the U/C standard curve.

10.3.1. Plasma

1.	Pipette **100 µL** of each **Plasma Standard**, **Plasma Control** and **patient plasma** into the respective **glass tubes.**
2.	Pipette **100 µL** of **Indicator Buffer** into each tube. Vortex.
3.	Pipette **20 µL** of **Acylation Reagent** into each tube. Vortex each tube immediately after pipetting.
4.	Cover tubes. **Incubate 30 min** at **RT (18-25°C).**
5.	Pipette **750 µL** of diluted **Assay Buffer** into each tube. Vortex.

10.3.2. Urine, Cell Culture Supernatants

1.	Pipette **50 µL** of each **U/C Standard**, **Urine Control** and **patient urine / cell culture sample** into the respective **glass tubes.**
2.	Pipette **50 µL** of **Indicator Buffer** into each tube. Vortex. If the indicator becomes colorless, the pH of the solution is too low and the sample contains too much acid. In that case add another 50 µL of Indicator Buffer until the solution remains reddish.
3.	Pipette **10 µL** of **Acylation Reagent** into each tube. Vortex each tube immediately after pipetting.
4.	Cover tubes. **Incubate 30 min** at **RT (18-25°C).**
5.	Pipette **2000 µL** of diluted **Assay Buffer** into each tube. Vortex thoroughly.

Note: The acylated samples can be stored at 2-8°C overnight or better at –20°C for up to 2 d.

11. TEST PROCEDURE

1.	Pipette **50 µL** of each acylated **Standard,** acylated **Control and** acylated **patient sample** into the respective wells of the Microtiter Plate.
2.	Pipette **50 µL** of freshly prepared **Enzyme Conjugate** into each well.
3.	Pipette **50 µL** of **Histamine Antiserum** into each well.
4.	Cover plate with adhesive foil. **Incubate 3 h** at **RT (18-25°C)** on an orbital shaker (500 rpm).
5.	Remove adhesive foil. Discard incubation solution. Wash plate **4 x** with **250 µL** diluted **Wash Buffer**. Remove excess solution by tapping the inverted plate on a paper towel.
6.	For adding of Substrate and Stop Solution use, if available, an 8-channel Micropipettor. Pipetting should be carried out in the same time intervals for Substrate and Stop Solution. Use positive displacement and avoid formation of air bubbles.
7.	Pipette **200 µL** of freshly prepared **TMB Substrate Solution** into each well.
8.	**Plasma: Incubate 40 min** at **RT (18-25°C)** on an orbital shaker (500 rpm). **Urine/Cell Culture Supernatants: Incubate 20 min** at **RT (18-25°C)** on an orbital shaker (500 rpm).
9.	Stop the substrate reaction by adding **100 µL** of **TMB Stop Solution** into each well. Briefly mix contents by gently shaking the plate.
10.	**Measure** optical density with a photometer at **450 nm** (Reference-wavelength: 600-650 nm) within **15 min** after pipetting of the Stop Solution.

12. QUALITY CONTROL

The test results are only valid if the test has been performed following the instructions. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable standards/laws. All standards and kit controls must be found within the acceptable ranges as stated on the QC Certificate. If the criteria are not met, the run is not valid and should be repeated. Each laboratory should use known samples as further controls.

In case of any deviation the following technical issues should be proven: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, incubation conditions and washing methods.

It is recommended to participate at appropriate quality assessment trials.

13. CALCULATION OF RESULTS

The obtained OD of the standards (y-axis, linear) are plotted against their concentration (x-axis, logarithmic) either on semi-logarithmic graph paper or using an automated method. A good fit is provided with cubic spline, 4 Parameter Logisitcs or Logit-Log.

For the calculation of the standard curve, apply each signal of the standards (one obvious outlier of duplicates might be omitted and the more plausible single value might be used).

The concentration of the samples can be read from the standard curve.

In case of diluted samples the values have to be multiplied with the corresponding dilution factor.

Samples showing concentrations above the highest standard have to be diluted as described in PRE-TEST SETUP INSTRUCTIONS and reassayed.

Calculate the 24 h excretion for each urine sample: µg/24h = µg/L x L/24h

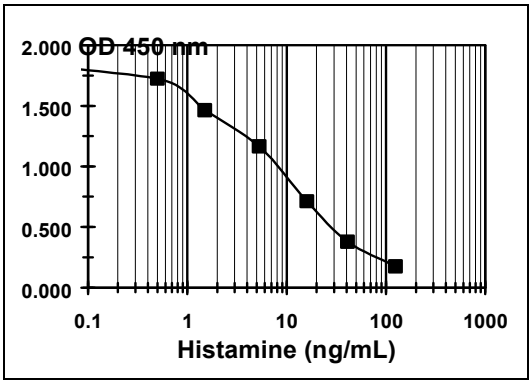
Conversion:

Histamine (ng/mL) x 8.997 = nmol/L

Typical Calibration Curve

(Example. Do not use for calculation!)

Standard	Histamine (ng/mL)	Mean OD	OD/OD_{max} (%)
A	0.0	1.929	100.0
B	0.5	1.723	89.3
C	1.5	1.462	75.8
D	5.3	1.165	60.4
E	16	0.712	36.9
F	41	0.380	19.7
G	125	0.174	9.0



14. EXPECTED VALUES

Apparently healthy subjects show the following values: (5 - 95 % percentile)

Plasma	Urine	
	24 h	spontaneous
ng/mL	µg/d	µg/g Creatinine
0.3 - 1.0	3.5 - 51	7.3 – 43.5

It is recommended that each laboratory establishes its own range of normal values.

15. LIMITATIONS OF THE PROCEDURE

Specimen collection has a significant effect on the test results. See SPECIMEN COLLECTION AND STORAGE for details. For cross-reactivities, see PERFORMANCE.
The following blood components do not have a significant effect (+/- 15 % of expected) on the test results up to the concentrations stated below:

Hemoglobin	5 mg/mL
Bilirubin	1 mg/mL
Triglyceride	30 mg/mL

16. PERFORMANCE

Analytical Specificity (Cross Reactivity)	Substance	Cross Reactivity (%)	Cross-reactivity of other substances tested < 0.005 %		
	N-Methyl-Histamine	2.42			
	N-Acetyl-Histamine	0.61			
	1-Methyl-Histamine	0.03			
Analytical Sensitivity (Limit of Detection)	Plasma	0.2 ng/mL	Mean signal (Zero-Standard) - 2SD		
	Urine	1.5 ng/mL			
Precision		Range (ng/mL)	CV (%)		
Intra-Assay	Plasma	0.23 – 83.7	2.4 – 14.9		
	Urine	5.4 – 145.4	1.9 – 4.9		
Inter-Assay	Plasma	6.6 – 85.5	7.0 – 12.9		
	Urine	6.3 – 152.8	7.1 – 11.9		
Linearity		Range (ng/mL)	Serial dilution up to	Range (%)	
	Plasma	10.7 – 33.0	1:8	79 - 126	
	Urine	52.6 - 132	1:32	82 - 107	
Recovery		Mean (%)	Range (%)	% Recovery after spiking	
	Plasma	103.0	92 - 118		
	Urine	101.0	95 - 108		
Method Comparison versus RIA	Plasma	GenWay -Assay = 0.83 x RIA + 0.45		r = 0.98; n = 90	
Method Comparison versus RIA	Urine	GenWay -Assay = 1.14 x RIA – 5.60		r = 0.93; n = 30	