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Instructions for use

Histamine ELISA

Enzyme Immunoassay for the determination of Histamine in plasma and urine.

For research use only, not for use in diagnostic procedures.

REF **IB89128**

Σ 96

+2 +8 °C

1. Introduction

1.1 Intended use and principle of the test

Enzyme Immunoassay for the determination of Histamine in plasma and urine. For research use only, not for use in diagnostic procedures.

In combination with the supplementary kit *Histamine Release* (Catalog # IB89145, sold separately), the assay can be used for the determination of histamine release in heparinized whole blood.
In the first part of the procedure, Histamine is acylated. The subsequent competitive ELISA kit uses the microtiter plate format. The antigen is bound to the solid phase of the microtiter plate. The acylated analyte concentrations in the standards, controls and samples and the solid phase bound analyte compete for a fixed number of antibody binding sites. After the system is in equilibrium, free antigen and free antigen-antibody complexes are removed by washing. The antibody bound to the solid phase is detected by an anti-goat IgG-peroxidase conjugate using TMB as a substrate. The reaction is monitored at 450 nm. Determination of unknown samples is achieved by comparing their absorbance with a standard curve prepared with known standard concentrations.

1.2 Background

Histamine belongs to the biogenic amines and is synthesized by decarboxylation from the amino acid histidine. It is synthesized by mast cells, basophils, platelets, histaminergic neurons, and enterochromaffine cells, where it is stored intracellularly in vesicles and released on stimulation.
Histamine acts by binding to its 4 receptors (H1R, H2R, H3R and H4R) on target cells in various tissues. It causes smooth muscle cell contraction, vasodilatation, increased vascular permeability and mucus secretion, tachycardia, alterations of blood pressure, and arrhythmias.
In humans, histamine is one of the most important mediators and takes part in the initial phase of an anaphylactic reaction ("immediate type" allergy).
Of interest is also the determination of the histamine *release* from basophilic leucocytes in allergies.

2. Procedural cautions, guidelines, warnings and limitations

2.1 Precautions, guidelines and warnings

(1) This kit is intended for professional use only. Users should have a thorough understanding of this protocol for the successful use of this kit. Only the test instruction provided with the kit is valid and has to be used to run the assay. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
(2) This assay was validated for certain types of sample as indicated in *Intended Use* (please refer to Chapter 1). Any off-label use of this kit is in the responsibility of the user and the manufacturer cannot be held liable.
(3) The principles of Good Laboratory Practice (GLP) have to be followed.
(4) In order to reduce exposure to potentially harmful substances, wear lab coats, disposable protective gloves and protective glasses where necessary.
(5) All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
(6) For dilution or reconstitution purposes, use deionized, distilled, or ultra-pure water.
(7) The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch with desiccant and used in the frame provided.
(8) Duplicate determination of sample is highly recommended to be able to identify potential pipetting errors.
(9) Once the test has been started, all steps should be completed without interruption. Make sure that the required reagents, materials and devices are prepared ready at the appropriate time.
(10) Incubation times do influence the results. All wells should be handled in the same order and time intervals.
(11) To avoid cross-contamination of reagents, use new disposable pipette tips for dispensing each reagent, sample, standard and control.
(12) A standard curve must be established for each run.
(13) The controls should be included in each run and fall within established confidence limits. The confidence limits are listed in the QC-Report provided with the kit.
(14) Do not mix kit components with different lot numbers within a test and do not use reagents beyond expiry date as shown on the kit labels.
(15) Avoid contact with Stop Solution containing 0.25 M H_2SO_4. It may cause skin irritation and burns. In case of contact with eyes or skin, rinse off immediately with water.
(16) TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them.
(17) For information on hazardous substances included in the kit please refer to Material Safety Data Sheet (MSDS). The Material Safety Data Sheet for this product is made available directly on the website of the manufacturer or upon request.

(18) The expected reference values reported in this test instruction are only indicative. It is recommended that each laboratory establishes its own reference intervals.
(19) The results obtained with this test kit are for research use only, not for use in diagnostic procedures.
(20) Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.

2.2 Limitations

Any inappropriate handling of samples or modification of this test might influence the results.

2.2.1 Interfering substances

Plasma

Samples containing precipitates or fibrin strands or which are haemolytic or lipemic might cause inaccurate results.

24-hour urine

Please note the sample preparation! If the percentage of the final concentration of acid is too high, the buffer capacity of the Acylation Buffer is insufficient. As a consequence histamine will not be acylated quantitatively.

2.2.2 Drug interferences

There are no known substances (drugs) which ingestion interferes with the measurement of histamine level in the sample.

2.2.3 High-Dose-Hook effect

No hook effect was observed in this test.

3. Storage and stability

Store the unopened reagents at 2 - 8 °C until expiration date. Do not use components beyond the expiry date indicated on the kit labels. Once opened the reagents are stable for 1 month when stored at 2 – 8 °C. Once the resealable pouch has been opened, care should be taken to close it tightly with desiccant again.

4. Materials

4.1 Contents of the kit

BA D-0090	FOILS	**Adhesive Foil** - Ready to use
Contents:	Adhesive Foils in a resealable pouch	
Volume:	1 x 4 foils	
BA E-0030	WASH-CONC 50x	**Wash Buffer Concentrate -** Concentrated 50x
Contents:	Buffer with a non-ionic detergent and physiological pH	
Volume:	1 x 20 ml/vial, light purple cap	
BA D-0024	REAC-PLATE	**Reaction Plate** - Ready to use
Contents:	1 x 96 well plate, empty in a resealable pouch	
BA E-1040	CONJUGATE	**Enzyme Conjugate** - Ready to use
Contents:	Rabbit anti-goat immunoglobulins conjugated with peroxidase	
Volume:	1 x 12 ml/vial, red cap	
BA E-0055	SUBSTRATE	**Substrate** - Ready to use
Contents:	Chromogenic substrate containing tetramethylbenzidine, substrate buffer and hydrogen peroxide	
Volume:	1 x 12 ml/black vial, black cap	

BA E-0080 STOP-SOLN **Stop Solution** - Ready to use

Contents: 0.25 M sulfuric acid

Volume: 1 x 12 ml/vial, light grey cap

BA E-1031 MTP HIS **Histamine Microtiter Strips** - Ready to use

Contents: 1 x 96 well (12x8) antigen precoated microwell plate in a resealable pouch with desiccant.

BA E-1010 HIS-AS **Histamine Antiserum** - Ready to use

Contents: Goat Anti- Histamine antibody, blue coloured

Volume: 1 x 12 ml/vial, blue cap

Standards and **Controls** - Ready to use

Cat. no.	Component	Colour/Cap	Concentration ng/ml	Concentration nmol/l	Volume/ Vial
BA E-1001	STANDARD A	white	0	0	4 ml
BA E-1002	STANDARD B	light yellow	0.5	4.5	4 ml
BA E-1003	STANDARD C	orange	1.5	13.5	4 ml
BA E-1004	STANDARD D	dark blue	5	45	4 ml
BA E-1005	STANDARD E	light grey	15	135	4 ml
BA E-1006	STANDARD F	black	50	450	4 ml
BA E-1051	CONTROL 1	light green	Refer to QC-Report for expected value and acceptable range!		4 ml
BA E-1052	CONTROL 2	dark red			4 ml

Conversion: Histamine (ng/ml) x 9 = Histamine (nmol/l)

Contents: Acidic buffer spiked with defined quantity of Histamine

BA E-1011 ACYL-BUFF **Acylation Buffer** - Ready to use

Contents: TRIS-buffer containing a non-mercury preservative

Volume: 1 x 4 ml/vial, pink cap

BA E-1012 ACYL-REAG **Acylation Reagent** - Lyophilized

Contents: Lyophilized acylation reagent

Volume: 2 vials, purple cap

BA E-0085 ACYL-SOLV **Acylation Solvent** - Ready to use

Contents: Ethanol

Volume: 1 x 5 ml/vial, brown cap

Hazards identification:

H225 Highly flammable liquid and vapour.

4.2 Additional materials and equipment required but not provided in the kit

- Calibrated precision pipettes to dispense volumes between 10 - 300 µl; 1.25 ml
- Microtiter plate washing device (manual, semi-automated or automated)
- ELISA reader capable of reading absorbance at 450 nm and if possible 620 - 650 nm
- Microtiter plate shaker (shaking amplitude 3 mm; approx. 600 rpm)
- Absorbent material (paper towel)
- Water (deionized, distilled, or ultra-pure)
- Vortex mixer

5. Sample collection and storage

In general the repeated freezing and thawing of samples should be avoided.

Plasma
Whole blood should be collected by venipuncture into centrifuge tubes containing EDTA as anti-coagulant (e.g. Monovette™ or Vacuette™ for plasma) and centrifuged at room temperature immediately after collection.
Haemolytic and especially lipemic samples should not be used for the assay.
Storage: up to 6 hours at 2 - 8 °C, for longer period (up to 6 month) at -20 °C.

Urine
Spontaneous urine or 24-hour urine, collected in a bottle containing 10 - 15 ml of 6 M HCl, can be used.
If 24-hour urine is used please record the total volume of the collected urine. If the percentage of the final concentration of acid is too high, the buffer capacity of the Diluent is insufficient. As a consequence interfering factors are not extracted quantitatively.
Storage: up to 6 hours at 2 - 8 °C, for longer periods (up to 6 month) at -20 °C.
Avoid exposure to direct sunlight.

Whole Blood
The release of histamine is performed with heparinized whole blood. For further information please refer to the instructions for use of the add-on kit **Histamine Release** (Catalog # IB89145, sold separately).

6. Test procedure

Allow all reagents and samples to reach room temperature and mix thoroughly by gentle inversion before use. Duplicate determinations are recommended.
The binding of the antibody and the enzyme conjugates and the activity of the enzyme used are temperature dependent, and the absorbance values may vary if a thermostat is not used. The higher the temperature, the higher the absorption values will be. Varying incubation times have similar influences on the absorbance. The optimal temperature during the Enzyme Immunoassay is between 20 - 25 °C.

⚠ *In case of overflow, read the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to 405 nm*

6.1 Preparation of reagents

Wash Buffer
Dilute the 20 ml Wash Buffer Concentrate with water (deionized, distilled or ultra-pure) to a final volume of 1000 ml.
Storage: 1 month at 2 – 8 °C

Acylation Solution
Reconstitute each vial of the Acylation Reagent (BA E-1012) with 2 ml Acylation Solvent (BA E-0085). Please make sure that it is completely dissolved before use.
If more than 2 ml are needed, pool the contents of the individual vials and mix thoroughly.
Storage: 1 month at 2 – 8 °C

6.2 Sample preparation and acylation

1.	Pipette **25 µl** of **standards**, **controls** and **plasma samples**, **10 µl** of **urine samples,** or **50 µl** of **supernatant** from the **release test*** into the respective wells of the **Reaction Plate.**
2.	Add **25 µl** of **Acylation Buffer** to all wells.
3.	Add **25 µl** of **Acylation Solution** (refer to 6.1) to all wells.
4.	Incubate for **45 min** at **RT** (20 - 25 °C) on a **shaker** (approx. 600 rpm).
5.	Add **100 µl** of **water** (deionized, distilled, or ultra-pure) to all wells.
6.	Incubate for **15 min** at **RT** (20 - 25 °C) on a **shaker** (approx. 600 rpm).
⚠	Take **25 µl of the prepared standards, controls and samples** for the **Histamine ELISA**

* For the **release test** the **Histamine Release** supplementary kit (Catalog # IB89145, sold separately) has to be used.

6.3 Histamine ELISA

1.	Pipette **25 µl** of the **acylated standards, controls** and **samples** into the appropriate wells of the **Histamine Microtiter Strips**.
2.	Pipette **100 µl** of the **Histamine Antiserum** into all wells and cover plate with **Adhesive Foil.**
3.	Incubate for **3 h** at **RT** (20 - 25 °C) on a **shaker** (approx. 600 rpm). ***Alternatively:*** *shake* the **Histamine Microtiter Strips** briefly by hand and incubate for **20 - 25 h** at **2 - 8 °C**.
4.	Remove the foil. Discard or aspirate the contents of the wells. Wash the plate **4 x** by adding **300 µl** of **Wash Buffer, discarding** the content and **blotting dry each time** by tapping the inverted plate on absorbent material.
5.	Pipette **100 µl** of the **Enzyme Conjugate** into all wells.
6.	Incubate for **30 min** at **RT** (20 - 25 °C) on a **shaker** (approx. 600 rpm).
7.	Discard or aspirate the contents of the wells. Wash the plate **4 x** by adding **300 µl** of **Wash Buffer, discarding** the content and **blotting dry each time** by tapping the inverted plate on absorbent material.
8. ⚠	Pipette **100 µl** of the **Substrate** into all wells and incubate for **20 - 30 min** at **RT** (20 - 25 °C) on a **shaker** (approx. 600 rpm). ***Avoid exposure to direct sunlight!***
9.	Add **100 µl** of the **Stop Solution** to each well and shake the microtiter plate to ensure a homogeneous distribution of the solution.
10.	**Read** the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to **450 nm** (if available a reference wavelength between 620 nm and 650 nm is recommended).

7. Calculation of results

		Histamine
Measuring range	Plasma	0.12 - 50 ng/ml
	Urine	0.3 – 125 ng/ml

The standard curve is obtained by plotting the absorbance readings (calculate the mean absorbance) of the standards (linear, y-axis) against the corresponding standard concentrations (logarithmic, x-axis). Use a non-linear regression for curve fitting (e.g. spline, 4- parameter, akima).

⚠ *This assay is a competitive assay. This means: the OD-values are decreasing with increasing concentrations of the analyte. OD-values found below the standard curve correspond to high concentrations of the analyte in the sample and have to be reported as being positive.*

Plasma samples and controls:
The concentrations of the **plasma samples** and the **controls** can be read directly from the standard curve.

Urine samples:
The read concentrations of **histamine in urine** have to be **multiplied by 2.5**

The total amount of Histamine excreted in urine during 24 h is calculated as following:

µg/24h = µg/l x l/24h

Conversion
Histamine (ng/ml) x 9 = Histamine (nmol/l)

Expected reference values
It is strongly recommended that each laboratory should determine its own reference values.

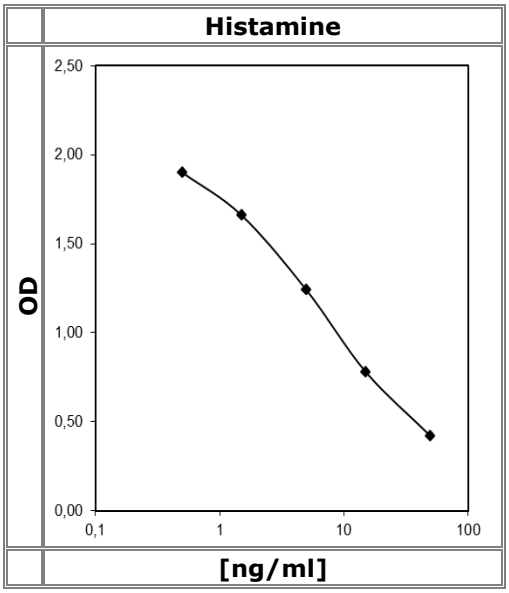
Plasma	24 hour-urine	Spontaneous urine
< 1 ng/ml	< 45 µg/d	< 45 µg/g creatinine

7.1 Quality control

It is recommended to use control samples according to national regulations. Use controls at both normal and elevated levels. The kit, or other commercially available, controls should fall within established confidence limits. The confidence limits of the kit controls are indicated in the QC-Report.

7.2 Typical standard curve

Example, do not use for calculation!



8. Assay characteristics

Analytical Sensitivity (Limit of Detection)	**Histamine**	
	Sensitivity Plasma	0.12 ng/ml
	Sensitivity Urine	0.30 ng/ml

	Substance	**Cross Reactivity (%)**
		Histamine
Analytical Specificity (Cross Reactivity)	Histamine	100
	3-Methyl-Histamine	0.1
	Tyramine	0.01
	L-Phenylalanine	< 0.001
	L-Histidine	< 0.001
	L-Tyrosine	< 0.001
	Tryptamine	< 0.001
	5-Hydroxy-Indole-Acetic Acid	< 0.001
	Serotonin	< 0.001

Precision							
Intra-Assay	Sample	Range (ng/ml)	CV (%)	**Inter-Assay**	Sample	Range (ng/ml)	CV (%)
Histamine Urine	1	9.7 ± 1.5	15	Histamine Control samples	1	9.9 ± 1.7	11.8
	2	18.6 ± 2.4	12.8		2	42.3 ± 3.4	7.9
Histamine Plasma	1	1.2 ± 0.2	15.8				
	2	5.0 ± 0.6	11.8				

Linearity		Range	Serial dilution up to	Range (%)
	Urine	4.33 - 70 ng/ml	1:16	90 - 124
	Plasma	0.74 – 8.48 ng/ml	1:16	85 -106

Recovery		Range ng/ml	Mean (%)	Range (%)
	Urine	6.9 – 25.9 ng/ml	115	113 - 117
	Plasma	0.36 – 6.5 ng/ml	84	78 - 89

Method comparison versus ELISA	Urine	ELISA = 0.9 ELISA (LDN) – 3.1	r = 0.98; n = 29
	Plasma	ELISA = 1.0 ELISA (LDN) – 0.4	r = 0.99; n = 47

9. References/Literature

(1) Yagci et al. TCTP/HRF pathway and angiogenesis: A feasible intercourse in chronic lymphocytic leukaemia. Leukemia Research, 37:665-670 (2013)

(2) Coulson et al. Paracetamol (acetaminophen) attenuates in vitro mast cell and peripheral blood mononucleocyte cell histamine release induced by N-acetylcysteine. Clinical Toxicology, 48(2):111-114 (2010)

(3) Rovere et al. Histamine and Selenium in Lung Cancer. Anticancer Research, 26: 2937-2942 (2006)

For orders, please contact:
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⚠ ***For updated literature or any other information please contact your local supplier.***

Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Expiry date	LOT	Batch code	I V D	For in-vitro diagnostic use only!
	Consult instructions for use	CONT	Content	CE	CE labelled
	Caution	REF	Catalogue number	RUO	For research use only!