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Instructions for use

Histamine ELISA

REF **IB89128R**

2°C – 8°C

Σ 96

RUO

For research use only – Not for use in diagnostic procedures

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1. Introduction

1.1 Intended use and principle of the test

Enzyme Immunoassay for the quantitative determination of Histamine in plasma and urine.
In combination with the supplementary kit *Histamine Release* (for details contact your local supplier), the assay can be used for the measurement of histamine release in heparinized whole blood.
In the first part of the procedure, Histamine is quantitatively 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.
Quantification 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).

2. Procedural cautions, guidelines, warnings and limitations

2.1 Procedural cautions, 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) The principles of Good Laboratory Practice (GLP) have to be followed.
(3) In order to reduce exposure to potentially harmful substances, wear lab coats, disposable protective gloves and protective glasses where necessary.
(4) 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.
(5) For dilution or reconstitution purposes, use deionized, distilled, or ultra-pure water.
(6) The microplate contains snap-off strips. Unused wells must be stored at 2 – 8 °C in the sealed foil pouch with desiccant and used in the frame provided.
(7) Duplicate determination of sample is highly recommended to be able to identify potential pipetting errors.
(8) 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.
(9) Incubation times do influence the results. All wells should be handled in the same order and time intervals.
(10) To avoid cross-contamination of reagents, use new disposable pipette tips for dispensing each reagent, sample, standard and control.
(11) A standard curve must be established for each run.
(12) 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.
(13) 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.
(14) 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.
(15) 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.
(16) For information on hazardous substances included in the kit please refer to Safety Data Sheet (SDS). The Safety Data Sheet for this product is made available directly on the website of the manufacturer or upon request.
(17) Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.
(18) In case of any severe damage to the test kit or components, the manufacturer has to be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components must not be used for a test run. They must be stored properly until the manufacturer decides what to do with them. If it is decided that they are no longer suitable for measurements, they must be disposed of in accordance with national regulations.

2.2 Limitations

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

2.2.1 Interfering substances and proper handling of specimens

Plasma

Samples containing precipitates or fibrin strands might cause inaccurate results. Hemolytic samples (up to 1 mg/ml hemoglobin), icteric samples (up to 50 mg/dl bilirubin) and lipemic samples (up to 1600 mg/dl triglycerides) have no influence on the assay results.

24-hour urine

Please note the sample preparation! If the percentage of the final concentration of acid is too high, this will lead to incorrect results for the urine samples.

2.2.2 Drug and food 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 kit and reagents at 2 – 8 °C until expiration date. Do not use kit and components beyond the expiry date indicated on the kit labels. Once opened, the reagents are stable for 2 months when stored at 2 – 8 °C. Once the resealable pouch of the ELISA plate has been opened, care should be taken to close it tightly again including the desiccant.

4. Materials

4.1 Contents of the kit

BA D-0024	**REAC-PLATE**	**Reaction Plate** – ready to use
Content:	1 x 96 well plate, empty, in a resealable pouch	
BA D-0090	**FOILS**	**Adhesive Foil** – ready to use
Content:	Adhesive foils in a resealable pouch	
Number:	1 x 4 foils	
BA E-0030	**WASH-CONC** **50x**	**Wash Buffer Concentrate** – concentrated 50x
Content:	Buffer with a non-ionic detergent and physiological pH	
Volume:	1 x 20 ml/vial, purple cap	
BA E-0055	**SUBSTRATE**	**Substrate** – ready to use
Content:	Chromogenic substrate containing 3,3’,5,5’-tetramethylbenzidine, substrate buffer and hydrogen peroxide	
Volume:	1 x 12 ml/vial, black cap	
BA E-0080	**STOP-SOLN**	**Stop Solution** – ready to use
Content:	0.25 M sulfuric acid	
Volume:	1 x 12 ml/vial, grey cap	
BA E-0085	**ACYL-SOLV**	**Acylation Solvent** – ready to use
Content:	Organic solvent	
Volume:	1 x 5 ml/vial, brown cap	
Hazard pictograms:	 GHS02 GHS07	
Signal word:	Danger	
BA E-1010	**HIS-AS**	**Histamine Antiserum** – ready to use
Content:	Goat anti-histamine antibody, in protein containing buffer, blue coloured	
Volume:	1 x 12 ml/vial, blue cap	
Description:	Species of the antibody is goat; species of the protein in the buffer is bovine	

BA E-1012	**ACYL-REAG**	**Acylation Reagent** – lyophilized
Content:	Lyophilized acylation reagent	
Volume:	2 vials, purple cap	
Hazard pictograms:	 GHS07	
Signal word:	Warning	
BA E-1031	**HIS**	**Histamine Microtiter Strips** – ready to use
Content:	1 x 96 wells (12x8) antigen precoated microwell plate in a resealable pouch with desiccant	
BA E-1040	**CONJUGATE**	**Enzyme Conjugate** – ready to use
Content:	Donkey anti-goat immunoglobulins conjugated with peroxidase	
Volume:	1 x 12 ml/vial, red cap	
Description:	Species is donkey	
Hazard pictograms:	 GHS07	
Signal word:	Warning	
Hazardous ingredients:	2-methyl-2H-isothiazol-3-one	
Hazard statements:	H317 May cause an allergic skin reaction.	
Precautionary statements:	P280 Wear protective gloves. P302+P352 IF ON SKIN: Wash with plenty of water. P333+P313 If skin irritation or rash occurs: Get medical advice/attention. P501 Dispose of contents/container to an authorised waste collection point.	
BA E-1311	**ACYL-BUFF**	**Acylation Buffer** – ready to use
Content:	TRIS buffer containing a non-mercury preservative	
Volume:	1 x 4 ml/vial, pink cap	

4.2 Calibration and Controls

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**	yellow	0.5	4.5	4 ml
BA E-1003	**STANDARD C**	orange	1.5	13.5	4 ml
BA E-1004	**STANDARD D**	blue	5	45	4 ml
BA E-1005	**STANDARD E**	grey	15	135	4 ml
BA E-1006	**STANDARD F**	black	50	450	4 ml
BA E-1051	**CONTROL 1**	green	Refer to QC-Report for expected value and acceptable range.		4 ml
BA E-1052	**CONTROL 2**	red			4 ml

Conversion: histamine [ng/ml] x 9 = histamine [nmol/l]

Content: Acidic buffer spiked with defined quantity of histamine.

4.3 Additional materials required but not provided in the kit

- Water (deionized, distilled, or ultra-pure)
- Absorbent material (paper towel)

4.4 Additional 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)
- Vortex mixer

5. Sample collection, handling 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 according to manufacturer's instructions at room temperature immediately after collection.
Hemolytic, icteric and lipemic samples should not be used for the assay.
Storage: up to 6 hours at 2 – 8 °C, for longer period (up to 6 months) at -20 °C.

When using gel collection tubes, the plasma must be collected immediately after centrifugation and frozen separately, otherwise there is a possibility of obtaining false positive results.

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.
Storage: up to 6 hours at 2 – 8 °C, for longer period (up to 6 months) 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** (for details contact your local supplier).

6. Test procedure

Allow all reagents and samples to reach room temperature and mix thoroughly by gentle inversion before use. Duplicate determinations are recommended. It is recommended to number the strips of the microwell plate before usage to avoid any mix-up.
The binding of the antisera and of the enzyme conjugate and the activity of the enzyme are temperature dependent. The higher the temperature, the higher the absorption values will be. Varying incubation times will have similar influences on the absorbance. The optimal temperature during the Enzyme Immunoassay is between 20 – 25 °C.

During the overnight incubation at 2 – 8 °C with the antiserum, the temperature should be uniform all over the ELISA plate to avoid any drift and edge-effect.

6.1 Preparation of reagents and further notes

Wash Buffer
Dilute the 20 ml Wash Buffer Concentrate **WASH-CONC** **50X** with water to a final volume of 1000 ml.
Storage: 2 months at 2 – 8 °C

Acylation Solution
Reconstitute each vial of the Acylation Reagent **ACYL-REAG** with 2 ml Acylation Solvent **ACYL-SOLV**. 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: 2 months at 2 – 8 °C

Histamine Microtiter Strips
In rare cases residues of the blocking and stabilizing reagent can be seen in the wells as small, white dots or lines. These residues do not influence the quality of the product.

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 **REAC-PLATE**.
2.	Add **25 µl** of **ACYL-BUFF** 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 (for details contact your local supplier) 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** **HIS**.
2.	Pipette **100 µl** of the **HIS-AS** into all wells and cover plate with **FOILS**.
3.	Incubate for **3 h** at **RT** (20 – 25 °C) on a **shaker** (approx. 600 rpm). ***Alternatively:*** *Shake* the **Histamine Microtiter Strips** **HIS** 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 **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-SOLN** 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	0.29 – 50 ng/ml
	Urine	0.96 – 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. 4-parameter, marquardt).

⚠ *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]

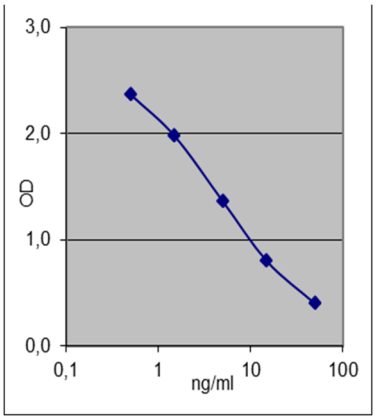
7.1 Expected reference value

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

Plasma	**24 hour-urine**	**Spontaneous urine**
0.2 – 1 ng/ml	5 – 56 µg/24h	8 – 53 µg/g creatinine

7.2 Typical standard curve

⚠*Example: Do not use for calculation!*



8. Control samples

The confidence limits of the kit controls are indicated on the QC-Report.

9. Assay characteristics

9.1 Performance data

Analytical Sensitivity Limit of Detection	
	Histamine
Sensitivity Plasma	0.18 ng/ml
Sensitivity Urine	0.22 ng/ml

Analytical Sensitivity Limit of Quantification	
	Histamine
Sensitivity Plasma	0.29 ng/ml
Sensitivity Urine	0.96 ng/ml

Analytical Specificity (Cross Reactivity)	
Substance	**Cross Reactivity [%]**
	Histamine
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				**Inter-Assay**			
	Sample	Range [ng/ml]	CV [%]		Sample	Range] [ng/ml]	CV [%]
Histamine Urine	1	9.7 ± 1.5	15	Histamine Urine	1	8.2 ± 0.9	11
	2	18.6 ± 2.4	13		2	12.8 ± 1.7	13
					3	42.2 ± 6.0	14
Histamine Plasma	1	1.2 ± 0.2	16	Histamine Plasma	1	0.29 ± 0.07	25
	2	5.0 ± 0.6	12		2	3.1 ± 0.2	8
					3	5.5 ± 0.8	15

Linearity			
	Serial dilution up to	Mean [%]	Range [%]
Urine	1:21	100	90 – 124
Plasma	1:10	101	85 – 106

Recovery			
	Range	Mean [%]	Range [%]
Urine	14.0 – 105 ng/ml	109	101 – 119
Plasma	0.4 – 6.5 ng/ml	84	78 – 89

Method comparison versus other RIA		
Urine	RIA = 0.9 ELISA – 3.1	r = 0.97; n = 29
Plasma	RIA = 1.0 ELISA – 0.4	r = 0.99; n = 49

10. References/Literature

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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
	Use-by date	LOT	Batch code		
	Consult instructions for use	CONT	Content		
	Caution	REF	Catalogue number		Distributor
	Date of manufacture			RUO	For research use only!