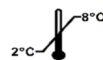


Instructions for use

Histamine ELISA

Please use only the valid version of the Instructions for Use provided with the kit

REF**BA E-1000R****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 urine and plasma to assess histamine balance.

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 to N-acyl histamine. The subsequent competitive ELISA uses the microtiter plate format. The antigen is bound to the solid phase of the microtiter plate. The acylated standards, controls and samples compete with the solid phase bound analytes 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 resulting in a colour reaction. The reaction is monitored at a wavelength of 450 nm.

Quantification of unknown samples is achieved by comparing their absorbance with a reference curve prepared with known standard concentrations. Manual processing of the ELISA is recommended. The use of automatic laboratory equipment is the responsibility of the user. This product is not intended to clinical diagnoses.

1.2 Background

Histamine is a biogenic amine and neurotransmitter and is formed from the amino acid L-histidine [1, 2]. It is synthesized and stored in mast cells and basophils until it is released upon appropriate stimulation and finally degraded by diamine oxidase and N-methyltransferase [2 – 4]. Histamine is involved in many mechanisms through its release, such as immunological, physiological, and inflammatory mechanisms, as well as smooth muscle contraction, vasodilation, and increased vascular permeability [2, 5 – 8].

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 must 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) must 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. For dilution or reconstitution purposes, use deionized, distilled, or ultra-pure water. Avoid repeated freezing and thawing of reagents and specimens.
- (5) 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. Microtiter strips which are removed from the frame for usage should be marked accordingly to avoid any mix up.
- (6) Duplicate determination of sample is highly recommended.
- (7) Once the test has been started, all steps should be completed without interruption. Make sure that the required reagents, materials, and devices are prepared for use at the appropriate time.
- (8) Incubation times do influence the results. All wells should be handled in the same order and time intervals.
- (9) To avoid cross-contamination of reagents, use new disposable pipette tips for dispensing each reagent, sample, standard and control.
- (10) A standard curve must be established for each run.
- (11) 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.
- (12) 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.
- (13) Avoid contact with Stop Solution containing 0.25 M H₂SO₄. It may cause skin irritation and burns. In case of contact with eyes or skin, rinse off immediately with water.
- (14) 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. Rinse contaminated items before reuse.
- (15) For information about 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.
- (16) Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.
- (17) 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

Urine

Please note the sample collection! It cannot be excluded that high acid concentrations lead to incorrect results.

Plasma

Samples containing precipitates or fibrin strands might cause inaccurate results.

Hemolytic samples (up to 1 mg/ml hemoglobin), icteric samples (up to 0.5 mg/ml bilirubin) and lipemic samples (up to 16 mg/ml triglycerides) have no influence on the assay results.

If the concentrations cannot be estimated and there are doubts as to whether the above limit values for hemolytic, icteric or lipemic samples are complied with, the samples should not be used in the assay.

2.2.2 Drug and food interferences

Foods rich in histamine and foods that promote histamine release should be avoided for 12 hours prior to sampling. These are mainly: alcoholic beverages, cheese, fruit, nuts, seafood and raw sausages. For a more detailed list of these foods, please contact a physician or the manufacturer.

Furthermore, certain medications (diamine oxidase inhibitors, histamine N-methyltransferase inhibitors) are able to influence histamine levels.

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-1011	ACYL-BUFF	Acylation Buffer – ready to use
Content:	Buffer with proteins and non-mercury preservative	
Volume:	1 x 4 ml/vial, pink cap	
Description:	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.	

4.2 Calibration and Controls

Standards and Controls – ready to use

Cat. no.	Component	Color/Cap	Concentration [ng/ml] HIS	Concentration [nmol/l] HIS	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 a 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 – 2000 µl
- 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

Repeated thawing and freezing of all samples should be avoided!

EDTA-Plasma

Whole blood should be collected by venipuncture into centrifuge tubes containing EDTA as anti-coagulant and centrifuged according to manufacturer's instructions at room temperature immediately after collection. 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. Hemolytic, icteric and lipemic samples should not be used for the assay.

Storage: up to 24 hours at 2 – 8 °C, for longer period (up to 6 months) at < -15 °C.

Spontaneous urine

Spontaneous urine should be collected in a sample cup, stabilized with 10 µl of 6 M HCl to 1 ml of urine. The measurement results are related to the creatinine content of the sample.

Storage: up to 24 hours at 18 – 25 °C, up to 5 days at 2 – 8 °C, for longer period (up to 6 months) at < -15 °C. Avoid exposure to direct sunlight.

24-hour urine

10 – 15 ml of 6 M HCl is placed in the collection container to stabilize the collected urine. For the quantitative determination of the amounts of histamine excreted in a day, it is necessary to determine the volume of the day's urine and to note it for the later evaluation of the results. The measurement results can also be related to the creatinine content of the sample.

Storage: up to 24 hours at 18 – 25 °C, up to 5 days at 2 – 8 °C, for longer period (up to 6 months) at < -15 °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. Number the microwell plates (Microtiter Strips which are removed from the frame for usage should be marked accordingly to avoid any mix-up). Duplicate determinations are recommended.

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.

⚠ *The use of a microtiter plate shaker with the following specifications is mandatory: shaking amplitude 3 mm; approx. 600 rpm. Shaking with differing settings might influence the results.*

⚠ *Do not exceed the temperature during the enzyme immunoassay of 20 – 25 °C and the prescribed incubation times. Too high temperature during the enzyme immunoassay and too long incubation times might influence the results.*

⚠ *To stop the acylation, deionized, distilled or ultra-pure water must be used in all cases. Otherwise, it may influence the results.*

⚠ *The addition of 10 µl of 6 M HCl to 1 ml of spontaneous urine must be strictly adhered to. If this amount of HCl deviates, the results may be influenced.*

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 **ACYL-REAG** (BA E-1012) with 2 ml **ACYL-SOLV** (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: 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 ACYL-BUFF to all wells.
3.	Add 25 µl Acylation Solution 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 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).
4.	Remove the FOILS . Discard or aspirate the contents of the wells. Wash the plate 4 times 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 each well.
6.	Incubate 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 times 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 each well 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 all wells and shake the microtiter plate shortly.
10.	Read the absorbance of the solution in the wells within 10 min, using a microtiter plate reader set to 450 nm (if available a reference wavelength between 620 nm and 650 nm is recommended).

7. Calculation of results

Measuring range	Histamine	
	Urine	0.91 – 125 ng/ml
	Plasma	0.32 – 50 ng/ml

The standard curve, which can be used to determine the concentration of the unknown samples, 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) using a concentration of 0.001 ng/ml for Standard A (this alignment is mandatory because of the logarithmic presentation of the data). Use 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.

Samples found with concentrations higher than the highest standard (Standard F) should be diluted accordingly with 0.1 M HCl and have to be re-assayed. For the calculation of the concentrations this dilution factor has to be taken into account.

Plasma samples and controls

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

Urine samples

The concentrations of the urine samples read from the standard curve must be **multiplied** by a factor of **2.5**.

Histamine related to the creatinine content of the sample: $\mu\text{g/g creatinine} = \frac{\mu\text{g histamine}}{\text{g creatinine}}$

The daily amount of histamine excreted in urine within 24 h is calculated as follows:

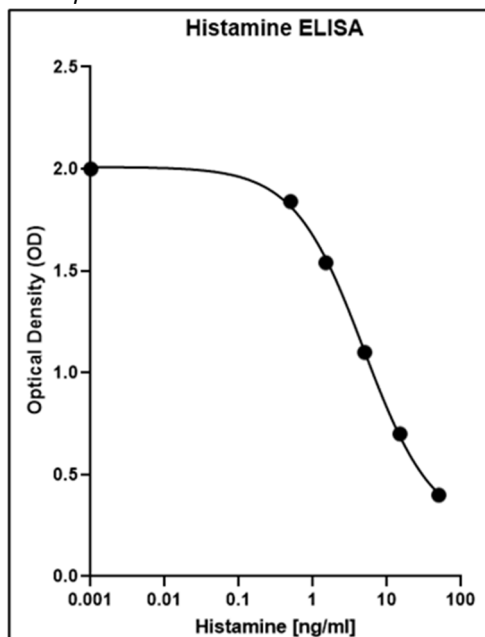
$\mu\text{g}/24\text{h} = \mu\text{g}/\text{l} \times \text{l}/24\text{h}$

Conversion:

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

7.1 Typical standard curve

⚠ Example. Do not use for calculation!



8. Control samples

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

9. Assay characteristics

9.1 Performance data

Analytical Sensitivity		
Limit of Blank (LOB)	Urine	0.19 ng/ml
	Plasma	0.12 ng/ml
Limit of Detection (LOD)	Urine	0.26 ng/ml
	Plasma	0.19 ng/ml
Limit of Quantification (LOQ)	Urine	0.91 ng/ml
	Plasma	0.32 ng/ml

Analytical Specificity (Cross Reactivity)	
Substance	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				Inter-Assay			
	Sample	Mean ± SD [ng/ml]	CV [%]		Sample	Mean ± SD [ng/ml]	CV [%]
Urine	1	9.7 ± 1.5	15.0	Urine	1	8.2 ± 0.94	11.4
	2	18.6 ± 2.4	12.8		2	12.8 ± 1.7	13.1
					3	42.2 ± 6.0	14.3
Plasma	1	1.2 ± 0.18	15.8	Plasma	1	0.78 ± 0.15	19.2
	2	5.0 ± 0.59	11.8		2	4.8 ± 0.36	7.6
					3	10.2 ± 0.79	7.7

Lot-to-Lot			
	Sample	Mean ± SD [ng/ml]	CV [%]
Histamine in artificial matrix (n = 6)	1	3.5 ± 0.4	10.7
	2	15.8 ± 1.1	6.7
Histamine in plasma (n = 6)	1	2.4 ± 0.5	19.4
	2	8.6 ± 0.8	8.9

Recovery			
	Range [ng/ml]	Mean [%]	Range [%]
Urine	3.7 – 126	113	105 – 127
Plasma	0.34 – 11.5	95.0	91.1 – 102

Linearity			
	Serial dilution up to	Mean [%]	Range [%]
Urine	1:64	130	122 – 135
Plasma	1:64	117	104 – 128

Method comparison (urine): ELISA vs. LC-MS/MS	LC-MS/MS = 0.8x – 3.2; r ² = 0.98; n = 35
Method comparison (plasma): ELISA vs. RIA	RIA = 1.4x + 0.65; r ² = 0.95; n = 37

9.2 Metrological Traceability

The values assigned to the standards and controls of the Histamine ELISA are traceable to SI Units by weighing with quality-controlled analyte.

Standards and Controls	Uncertainty [%]
	2.5

Histamine ELISA		
Urine	Concentration [ng/ml]	Expanded Uncertainty [%] k = 2*
	8.2	23.3
	12.8	26.7
	42.2	29.0
Plasma	Concentration [ng/ml]	Expanded Uncertainty [%] k = 2*
	0.78	38.7
	4.8	16.0
	10.2	16.2

* This defines an interval about the measured result that will include the true value with a probability of 95%.

10. References/Literature

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For updated literature or any other information please contact your local supplier.

11. Changes

Version	Release Date	Chapter	Change
18.0-r	2022-05-02	1. 2.1 2.2.2 5. 6.3 7. 9.1 9.2 10.	– Introduction – Procedural notes, guidelines and warnings – Drug and food interferences – Sample collection and storage – Alternative antiserum incubation overnight was removed – Measuring range, expected reference value and typical standard curve have been updated – Performance data updated and Lot-to-Lot added – Metrological traceability added – References/Literature updated
19.0-r	2023-02-10	6 6.1 7.1 9.1	– New warning notices included – Acylation Solution: Shelf life after opening 2 months – Typical standard curve updated – Recovery updated
20.0-r	2024-07-16	4.1 9.1 9.2	– Hazard labelling updated according to SDS – Lot-to-Lot updated – Metrological Traceability updated

Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Use-by date		Batch code		
	Consult instructions for use		Content		
	Caution		Catalogue number		Distributor
	Date of manufacture				For research use only!