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LDN®

Instructions for use

Histamine ELISA

REF **BA E-1000**

Σ 96

+2 +8 °C

IVD

CE

Histamine ELISA

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* (REF BA E-1100), 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 standards, controls and samples and the solid phase bound analyte compete for a fixed number of antiserum binding sites. After the system is in equilibrium, free antigen and free antigen-antiserum complexes are removed by washing. The antibody bound to the solid phase is detected by an anti-rabbit 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 reference curve prepared with known standard concentrations.

2. Procedural Cautions, Guidelines and Warnings

- This kit is 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.
- This assay was validated for a certain type 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 can not be taken liable.
- Reagents of kits which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
- The principles of Good Laboratory Practice (GLP) have to be followed.
- In order to reduce exposure to potentially harmful substances, wear lab coats, disposable latex gloves and protective glasses where necessary.
- 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.
- When the use of water is specified for dilution or reconstitution, use deionized, distilled, or ultra-pure water.
- The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch and used in the frame provided.
- It is highly recommended to determine samples in duplicate to be able to identify potential pipetting errors.
- 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.
- Incubation times do influence the results. All wells should be handled in the same order and time sequences.
- To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard and control.
- A calibrator curve must be established for every run.
- The controls should be included in every run and fall within established confidence limits. The valid confidence limits are listed in the QC-report included in the kit.
- Do not mix various lot numbers of kit components within a test and do not use reagents beyond expiry date as shown on the kit labels.
- 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, flush immediately with water.
- 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.
- 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.
- For information on hazardous substances included in the kit please refer to Material Safety Data Sheets (MSDS). The Material Safety Data Sheet for this product is available directly on the website of the manufacturer or upon request.
- The expected reference values reported in this test instruction are only indicative. It is recommended that each laboratory establishes its own reference intervals.
- The results obtained with this test kit should not be taken as the sole reason for any therapeutic consequence but have to be correlated to other diagnostic tests and clinical observations.

- Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.
- In case of complaints please submit to the manufacturer a written report (the corresponding form is available upon request) containing all data as to how the test was conducted, the results received and a copy of the original test printout.

3. Storage and stability

Store the reagents at 2 - 8 °C until expiration date. Do not use components beyond the expiry date indicated on the kit labels. Do not mix various lots of any kit component within an individual assay.

4. Materials

4.1 Contents of the kit

REF	Symbol	Reagent	Content	Colour Code	
BA D-0024	REAC-PLATE	**Reaction Plate**	1 x 96 wells		ready for use
BA D-0090	FOILS	**Adhesive Foil**	1 x 4		ready for use
BA E-0030	WASH-CONC 50x	**Wash Buffer Concentrate**	1 x 20 ml	light purple	concentrated
BA E-0055	SUBSTRATE	**Substrate**	1 x 12 ml	black	ready for use, containing a solution of TMB
BA E-0080	STOP-SOLN	**Stop Solution**	1 x 12 ml	light grey	ready for use
BA E-1001	STANDARD A	**Standard A**	1 x 4 ml	white	ready for use
BA E-1002	STANDARD B	**Standard B**	1 x 4 ml	light yellow	ready for use
BA E-1003	STANDARD C	**Standard C**	1 x 4 ml	orange	ready for use
BA E-1004	STANDARD D	**Standard D**	1 x 4 ml	dark blue	ready for use
BA E-1005	STANDARD E	**Standard E**	1 x 4 ml	light grey	ready for use
BA E-1006	STANDARD F	**Standard F**	1 x 4 ml	black	ready for use
BA E-1010	HIS-AS	**Histamine Antiserum**	1 x 12 ml	blue	ready for use, from goat
BA E-1011	ACYL-BUFF	**Acylation Buffer**	1 x 4 ml	pink	ready for use
BA E-1012	ACYL-REAG	**Acylation Reagent**	4 vials	purple	lyophilised
BA E-1031	HIS	**Histamine Microtiter Strips**	1 x 96 wells		12 strips, 8 wells each, break apart, pre-coated
BA E-1040	CONJUGATE	**Enzyme Conjugate**	1 x 12 ml	red	ready for use, anti-goat IgG conjugated with peroxidase
BA E-1051	CONTROL 1	**Control 1**	1 x 4 ml	light green	ready for use
BA E-1052	CONTROL 2	**Control 2**	1 x 4 ml	dark red	ready for use
BA E-0085	ACYL-SOLV	**Acylation Solvent**	1 x 10 ml	brown	ready for use

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
- 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
Plasma (EDTA, Heparin) should be used. Haemolytic and especially lipemic samples should not be used with this assay.
Storage: up to 6 hours at 2 - 8 °C; for longer periods (up to 6 months) at -20 °C.

Urine
Spontaneous or 24-hour urine may be used.
Storage: up to 6 hours at 2 - 8 °C; for longer periods (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** (REF BA E-1100).

6. Test procedure

Allow all reagents and samples to reach room temperature and mix thoroughly by gentle inversion before use. Duplicate determinations are recommended.

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: up to 6 months 2 - 8 °C

Acylation Solution
The Acylation Solution has to be prepared freshly prior to the assay:
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.

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 (REF BA E-1100) 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

	Concentration of the standards					
Standard	**A**	**B**	**C**	**D**	**E**	**F**
Histamine (ng/ml = µg/l)	0	0.5	1.5	5	15	50
Histamine (nmol/l)	0	4.5	13.5	45	135	450
Conversion:	Histamine (ng/ml) x 9 = Histamine (nmol/l)					

The calibration 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).

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

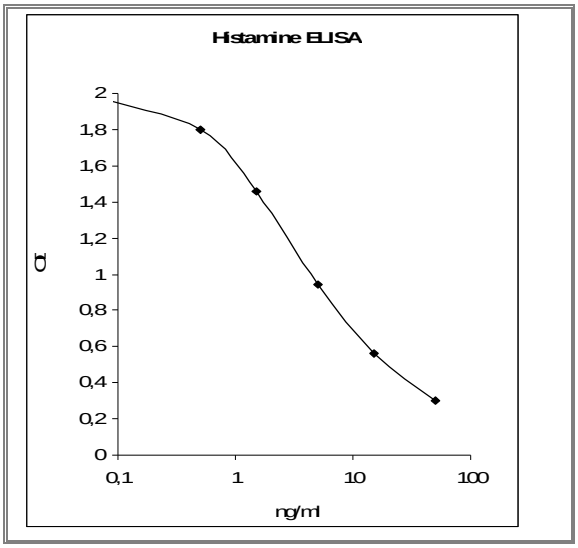
7.1 Quality control
It is recommended to use control samples according to national regulations. Use controls at both normal and pathological levels. The kit, or other commercially available, controls should fall within established confidence limits. The confidence limits of the kit controls are indicated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

7.2 Calibration
The binding of the antisera and the enzyme conjugates and the activity of the enzyme used are temperature dependent, and the extinction values may vary if a thermostat is not used. The higher the temperature, the higher the extinction values will be. The extinction values also depend on the incubation times. 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*

7.3 Typical calibration curve

⚠ *Example, do not use for calculation!*



8. Assay characteristics

Expected Reference Values	**Histamine**		
	Plasma	Urine	
		24 h	spontaneous
	< 1 ng/ml	< 45 µg/d	< 45 µg/g creatinine

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 (%)
	Histamine	Urine	4.33 - 70 ng/ml	1:16	90 - 124
		Plasma	0.74 – 8.48 ng/ml	1:16	85 -106

			Mean (%)	Range (%)	% Recovery after spiking
Recovery	Histamine	Urine	115	113 - 117	
		Plasma	84	78 - 89	

Method comparison versus ELISA	Histamine	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

⚠ ***For updated literature, information about clinical significance 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!