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LDN®

Instructions for use

Histamine ELISA Fast Track

REF BA E-1700

96 +2 +8 °C IVD CE

Histamine ELISA (Cell culture, Urine and Whole blood)

1. Intended use and principle of the test

Enzyme Immunoassay for the quantitative determination of Histamine in urine and cell culture samples and for the quantitative determination of Total Histamine in whole blood.

First, 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. Advice on handling the test

2.1 Reliability of the test results

In order to assure a reliable evaluation of the test results it must be conducted according to the instructions included and in accordance with current rules and guidelines (GLP, RILIBÄK, etc.). Special attention must be paid to control checks for precision and correctness during the test; the results of these control checks have to be within the norm range. In case of significant discrepancies between the pre-set assay characteristics of this test and the actual results please contact the manufacturer of the test kit for further instructions.
It is recommended that each laboratory establishes its own reference intervals. The values reported in this test instruction are only indicative.
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.

2.2 Complaints

In case of complaints please submit to the manufacturer a written report containing all data as to how the test was conducted, the results received and a copy of the original test printout. Please contact the manufacturer to obtain a reclamation form and return it completely filled in to the manufacturer.

2.3 Warranty

This test kit was produced according to the latest developments in technology and subjected to stringent internal and external quality control checks. Any alteration of the test kit or the test procedure as well as the usage of reagents from different charges may have a negative influence on the test results and are therefore not covered by warranty. The manufacturer is not liable for damages incurred in transit.

2.4 Disposal

Residual substances and/or all remaining chemicals, reagents and ready for use solutions, are special refuse. The disposal is subject to the laws and regulations of the federation and the countries. About the removal of special refuse the responsible authorities or refuse disposal enterprises inform. The disposal of the kit must be made according to the national official regulations. Legal basis for the disposal of special refuse is the cycle economic- and waste law.
The appropriate safety data sheets of the individual products are available on the homepage. The safety data sheets correspond to the standard: ISO 11014-1.

2.5 Interference

Do not mix reagents and solutions from different lots. Consider different transport and storage conditions. Inappropriate handling of test samples or deviations from the test regulation can the results affect. Use no kit components beyond the expiration date. Avoid microbiological contamination of the reagents and the washing water. Consider incubation periods and wash references.

2.6 Precautions

Observe the incubation periods and washing instructions. Never pipette by mouth and avoid contact of reagents and specimens with skin. No smoking, eating or drinking in areas where samples or kit test tubes are handled. When working with kit components or samples, always wear protective gloves and wash your hand thoroughly as soon as you have finished the work. Avoid spraying of any kind. Avoid any skin contact with reagents. Use protective clothing and disposable gloves. All steps have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes. Sodium azide could react with lead and copper tubes and may form highly explosive metal azide. When clearing up, rinse thoroughly with large volumes of water to prevent such formation.
All reagents of this testkit which contain human or animal 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.

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.

4.1 Contents of the kit

BA D-0024	REAC-PLATE	**Reaction Plate**	1 x 96 wells	ready for use
BA E-0030	WASH-CONC 50x	**Wash Buffer Concentrate**	1 x 20 mL	concentrate, dilute content with dist. water to a final volume of 1000 mL
BA E-0041	DILUENT	**Diluent**	1 x 22 mL	ready for use
BA E-0055	SUBSTRATE	**Substrate**	1 x 12 mL	ready for use, containing a solution of tetramethylbenzidine (TMB)
BA E-0080	STOP-SOLN	**Stop Solution**	1 x 12 mL	ready for use, containing 0.25 M H_2SO_4.
BA E-1031	HIS	**Histamine Microtiter Strips**	1 x 96 wells	12 strips, 8 wells each, break apart, precoated
BA E-1001	STANDARD A	**Standard A**	1 x 4 mL	ready for use
BA E-1002	STANDARD B	**Standard B**	1 x 4 mL	ready for use
BA E-1003	STANDARD C	**Standard C**	1 x 4 mL	ready for use
BA E-1001	STANDARD D	**Standard D**	1 x 4 mL	ready for use
BA E-1005	STANDARD E	**Standard E**	1 x 4 mL	ready for use
BA E-1006	STANDARD F	**Standard F**	1 x 4 mL	ready for use
BA E-1051	CONTROL 1	**Control 1**	1 x 4 mL	ready for use
BA E-1052	CONTROL 2	**Control 2**	1 x 4 mL	ready for use
BA E-1210	HIS-AS	**Histamine Antiserum**	1 x 12 mL	from goat, ready for use
BA E-1211	ACYL-BUFF	**Acylation Buffer**	2 x 12 mL	ready for use
BA E-1212	ACYL-REAG	**Acylation Reagent**	2 x 1,5 ml	ready for use
BA E-1240	CONJUGATE	**Enzyme Conjugate**	1 x 12 ml	ready for use, anti-goat IgG conjugated with peroxidase

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

- Calibrated variable precision micropipettes (e.g. 10-100 µL / 100-1000 µL)
- Microtiter plate washing device
- ELISA reader capable of reading absorbance at 450 nm
- Absorbent material (paper towel)
- Distilled water
- Vortex mixer

⚠For the determination of total histamine in Whole Blood, a Release Buffer is necessary!
(Please ask your local supplier.)

BA E-1726	RELEASE-BUFF	**Release Buffer**	1 x 250 mL	ready for use

⚠The assay can be performed with or without the usage of a shaker. If a shaker is used, it should have the following characteristics: shaking amplitude 3mm; capable of approx. 600 rpm.

5. Sample collection and storage

Cell culture
Samples can be stored at 2 – 8 °C for up to 6 hours. For longer periods (≤ 6 months) the samples should be stored between –20 °C and -80 °C.

Urine
Spontaneous urine or 24-hour urine, collected in a bottle containing 10-15 mL of 6 M HCl, may be used.
Storage: up to 6 hours at 2 - 8°C; for longer periods (up to 6 months) at -20°C.
Repeated freezing and thawing should be avoided.
Avoid exposure to direct sunlight.

Whole Blood
It may only be used heparinized whole blood.

Storage:	20-25°C	Keep away from heat or direct sun light.
Stability:	24 h	Avoid cooling. At 2-8°C, the leucocytes will clot.

6. Test procedure

Allow all reagents 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 distilled water to a final volume of 1000 mL.
Storage: up to 6 months 4–8°C

Acylation Reagent
The Acylation Diluent has a freezing point of 18.5°C. To ensure that the Acylation Diluent is liquid when being used, it must be ensured that the Acylation Diluent has reached room temperature and forms a homogeneous, crystal-free solution before being used. Alternative the Acylation Diluent can be stored at room temperature (20 – 25°C) separate from the other kit components.

Whole Blood
For the determination of total Histamine in whole blood, dilute heparinized whole blood 1+20 with **Release Buffer** (BA E-1726) and incubate for **10 min** at **90°C.**
(e.g. 50 µL whole blood plus 1 mL Release Buffer.)
Incubate **10 min** at **2 – 8°C**, to let the samples cool down.
Centrifuge for 10 min at 700 x g (brake switched-off)

6.2 Sample preparation and acylation

1.	Pipette **100 µL** of **standards**, **100 µL** of **controls** and **20 µL** of **cell culture samples** or **urine samples** and **100 µL** of perpetrated **whole blood samples** (refer to 6.1.*)* into the respective wells of the **Reaction Plate**.
2.	Add **80 µL** of **Diluent** to the wells with **cell culture samples** and **urine samples**.
3.	Add **25 µL** of **Acylation Reagent** (refer to 6.1*)* to all wells.
3.	Pipette **200 µL** of **Acylation Buffer** into all wells.
4.	Incubate **15 minutes** at **RT** (20-25°C) on a shaker (approx. 600 rpm) ***Alternatively without shaker:*** *shake* ***the Reaction Plate*** *shortly by hand and incubate* ***15 min at RT*** *(20-25°C).*
⚠	**Take 25 µL for the subsequent ELISA**

6.3 Histamine ELISA

1.	Pipette **25 µL** of the **acylated standards, controls and samples** into the wells of the **Histamine Microtiter Strips**.
2.	Pipette **100 µL** of the **Histamine Antiserum** into all wells.
3.	Incubate **30 min** at **RT** (20-25°C) on a shaker (approx. 600 rpm). ***Alternatively without shaker:*** *shake the* ***Histamine Microtiter Strips*** *shortly by hand and incubate for* ***40 min*** *at* ***RT*** *(20-25°C).*
4.	Discard or aspirate the contents of the wells and **wash** each well **3 times** thoroughly with **300 µL Wash Buffer**. Blot dry by tapping the inverted plate on absorbent material.
5.	Pipette **100 µL** of the **Enzyme Conjugate** into all wells.
6.	Incubate for **10 min** at **RT** (20-25°C) on a shaker (approx. 600 rpm). ***Alternatively without shaker:*** *incubate for* ***20 min*** *at* ***RT*** *(20-25°C).*
7.	Discard or aspirate the contents of the wells and **wash** each well **3 times** thoroughly with **300 µL Wash Buffer**. Blot dry by tapping the inverted plate on absorbent material.
8.	Pipette **100 µL** of the **Substrate** into all wells.
9.	Incubate for **15 ± 2 min** at **RT** (20-25°C) on a **shaker** (approx. 600 rpm).
⚠	***Alternatively without shaker:*** *incubate for* **15 ± 2** ***min*** *at* ***RT*** *(20-25°C).* ***Avoid exposure to direct sun light!***
10.	Add **100 µL** of the **Stop Solution** to each well and shake the microtiter plate shortly by hand to ensure a homogeneous distribution of the solution.
11.	**Read** the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to **450 nm** and a reference wavelength between 620 nm and 650 nm.

7. Calculation of results

	Concentration of the standards					
Standard	**A**	**B**	**C**	**D**	**E**	**F**
Histamine (ng/mL)	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).

Controls:
The concentrations of the **controls** can be read directly from the standard curve.

Urine samples:
The read concentrations have to be **multiplied by 5.**

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

Cell culture samples:
The read concentrations have to be **multiplied by 5**.

Whole blood samples:
The read concentrations of the **samples** have to be **multiplied by 21.**

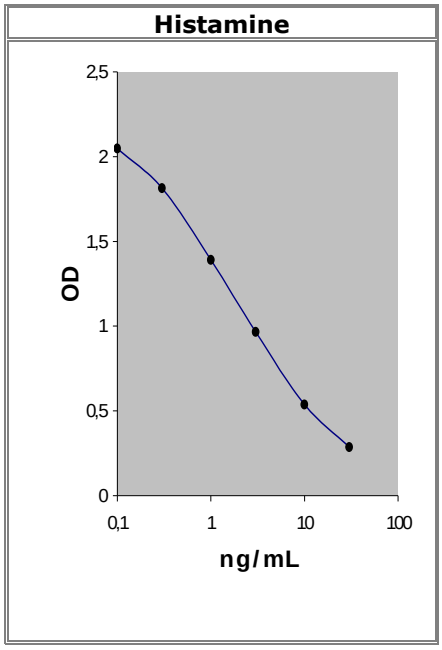
7.1 Quality control
It is recommended to use control samples according to state and federal regulations. Use controls at both normal and pathological levels. The kit or other commercially available controls should fall within established confidence limits. The confidence limits of the kit controls are indicated on 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**		
	Whole Blood	Urine	
	Total Histamine	24 h	spontaneous
	20 - 200 ng/mL	< 45 µg/d	< 70 µg/g creatinine

Analytical Specificity (Cross Reactivity)	**Substance**	**Cross Reactivity (%)**
		Histamine
	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

Analytical Sensitivity (Limit of Detection)	Histamine	0.2 ng/mL	Mean signal (Zero-Standard) - 2SD

Precision					
Inter-Assay Variation, n = 13			**Intra-Assay Variation**, n = 39		
Sample	Mean ± SD (ng/mL)	CV (%)	Sample	Mean ± SD (ng/mL)	CV (%)
1	2.03 ± 0.16	8	1	0.6 ± 0.1	12
2	6.74 ± 0.37	5.6	2	4.6 ± 0.3	6.3

Linearity		Range (ng/mL)	Range (%)	Mean (%)
	Histamine	0.74 - 8.48	85 - 106	100

Recovery		Serial dilution up to	Range (%)	Mean (%)
	Histamine	1:16	92 - 120	103

⚠ ***For current 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!