Distribuito in ITALIA da
Li StarFish S.r.l.
Via Cavour, 35
20063 Cernusco S/N (MI)
telefono 02-92150794
info@listarfish.it
www.listarfish.it

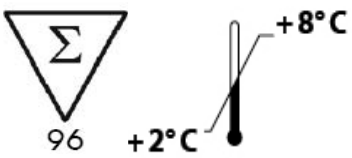
Manual

Histamine ELISA

For the in vitro determination of histamine in EDTA plasma and urine

Valid from 2019-07-17

REF **K 8212**



IVD CE

Immundiagnostik AG, Stubenwald-Allee 8a, 64625 Bensheim, Germany
Tel.: + 49 6251 70190-0 Fax: + 49 6251 70190-363
e.mail: info@immundiagnostik.com www.immundiagnostik.com

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1. INTENDED USE

This Immundiagnostik AG assay is intended for the quantitative determination of histamine in EDTA plasma and urine. For *in vitro* diagnostic use only.

2. INTRODUCTION

Histamine is a biogenic amine that derives from the decarboxylation of histidine. It is synthesised in mast cells, basophils, platelets, histaminergic neurons and enterochromaffine cells, where it is stored in vesicles. After stimulation and release, 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, vasodilation, increased vascular permeability and mucous 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).

The quantification of histamine release from basophilic leucocytes in allergies is also of clinical interest.

3. MATERIAL SUPPLIED

Cat. No.	Label	Kit Components	Quantity
K 8212	PLATE	Microtiter plate, pre-coated	12 x 8 wells
K 8212	STD	Standards, ready-to-use (0, 0.3, 0.6, 1.2, 5, 25 ng/ml)	6 x 2 ml
K 8212	CTRL 1	Control, ready-to-use (see specification for range)	1 x 2 ml
K 8212	CTRL 2	Control, ready-to-use (see specification for range)	1 x 2 ml
K 0006.C.100	WASHBUF A	Wash buffer concentrate, 10x	2 x 100 ml
K 8212	AB	Histamine antibody, peroxidase-labelled, ready-to-use	1 x 6 ml
K 8212	REABUF	Reaction buffer, ready-to-use	1 x 30 ml
K 8212	DER	Derivatisation reagent, lyophilised	1 vial
K 0008.10	DMSO	Dimethylsulfoxide (DMSO)	1 x 10 ml

K 0002.15	SUB	Substrate (tetramethylbenzidine), ready-to-use	1 x 15 ml
K 0003.15	STOP	Stop solution, ready-to-use	1 x 15 ml

For reorders of single components, use the catalogue number followed by the label as product number.

4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultrapure water*
- Calibrated precision pipets and 10-1000 µl single-use tips
- Foil to cover the microtiter plate
- Horizontal microtiter plate shaker
- Multi-channel pipets or repeater pipets
- Vortex
- Centrifuge
- Standard single-use laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader (required filters see chapter 7)

* Immundiagnostik AG recommends the use of ultrapure water (water type 1; ISO 3696), which is free of undissolved and colloidal ions and organic molecules (free of particles > 0.2 µm) with an electrical conductivity of 0.055 µS/cm at 25 °C (≥18.2 MΩ cm).

5. STORAGE AND PREPARATION OF REAGENTS

- To run the assay more than once ensure that reagents are stored at the conditions stated on the label.
- **Preparation of the wash buffer**: The **wash buffer concentrate (WASHBUF A)** has to be diluted with ultrapure water **1:10** before use (100 ml WASHBUF A + 900 ml ultrapure water), mix well. Crystals could occur due to high salt concentration in the concentrate. Before dilution, the crystals have to be redissolved at room temperature or in a water bath at 37 °C. The **WASHBUF A** is stable at **2-8 °C** until the expiry date stated on the label. **Wash buffer** (1:10 diluted WASHBUF A) can be stored in a closed flask at **2-8 °C for 1 month**.
- **DMSO** crystallises at 2-8 °C. Before use, dissolve the crystals at room temperature or in a water bath.
- The lyophilised **derivatisation reagent (DER)** is stable at **2-8 °C** until the expiry date stated on the label. Bring to room temperature before opening and

dissolve the content of the vial in **DMSO** as stated on the label. Allow to dissolve for **15 min** and mix thoroughly with a vortex-mixer.

The derivatisation reagent (reconstituted DER) **can be stored at 2-8 °C for 2 months**. Bring to room temperature before reuse. Please note: DMSO attacks all plastics but not polypropylene products and laboratory glass.

- All other test reagents are ready-to-use. Test reagents are stable until the expiry date (see label) when stored at **2-8 °C.**

6. STORAGE AND PREPARATION OF SAMPLES

EDTA plasma

- Collect blood into a chilled tube containing EDTA only, mix gently by inversion and chill immediately on ice. Centrifuge 10 minutes at 900 *g* at 4 °C within 20 minutes of sample collection.
- Lipemic or hemolytic samples may give erroneous results and should not be used for analysis.
- EDTA plasma samples are used **undiluted.**

Urine

- It is possible to use spontaneous as well as 24-h urine. 24-h urine: The total volume of urine excreted during 24 h is collected in a bottle containing 10-15 ml of 6 M HCl as preservative. Avoid exposure to direct sunlight. Determine total volume for calculation of results.
- Urine samples are **diluted 1:15** for derivatisation (see sample preparation procedure).
- Samples with visible amounts of precipitates should be centrifuged.

For sample preparation, a derivatisation reagent (DER) for derivatisation of histamine is added (see sample preparation procedure).

Sample Storage

Freshly collected EDTA plasma can be stored for up to 6 hours at 2-8 °C. For longer storage keep plasma samples frozen at -20 °C. Avoid repeated thawing and freezing.

Spontaenous urine is stable for up to 6 hours at 2-8 °C. Acidified urine samples can be stored at 2-8 °C up to 72 h. For longer periods, store urine samples frozen at -20 °C. Avoid repeated thawing and freezing.

7. ASSAY PROCEDURE

Principle of the test

This ELISA is designed for the quantitative determination of histamine. This assay is based on the method of competitive enzyme linked immunoassays.

The sample preparation includes the addition of a derivatisation reagent for histamine derivatisation. Afterwards, the treated samples and a peroxidase-conjugated polyclonal histamine antibody are incubated in wells of a microtiter plate coated with histamine derivative (tracer). During the incubation period, the target histamine in the sample competes with the tracer, immobilized on the wall of the microtiter wells, for the binding of the polyclonal antibodies.

After washing away the unbound components tetramethylbenzidine (TMB) is added as a peroxidase substrate. Finally, the enzymatic reaction is terminated by an acidic stop solution. The colour changes from blue to yellow, and the absorbance is measured in a photometer at 450 nm. The intensity of the yellow colour is inverse proportional to the histamine concentration in the sample; this means, high histamine concentration in the sample reduces the concentration of tracer-bound antibody and lowers the photometric signal. A dose response curve of the absorbance unit (optical density, OD at 450 nm) vs. concentration is generated, using the values obtained from the standards. Histamine, present in the patient samples, is determined directly from this curve.

Sample preparation procedure

Bring **all reagents and samples to room temperature** (15-30 °C) and mix well.

For **EDTA plasma** samples **no dilution** is required.

Dilute **urine samples** with reaction buffer by **factor 1:15**, as follows:

25 µl urine sample + **350 µl** reaction buffer (REABUF).

Derivatisation of standards, controls, plasma samples and diluted urine samples is carried out in vials (e.g. 1.5 ml polypropylene vials).

We recommend preparing one derivatisation per standard, control and sample and transferring it in duplicate determinations into the wells of the microtiter plate.

1.	Add **75 µl standard** (STD)/**control** (CTRL)/**plasma sample** or **diluted urine sample** in the corresponding vials.

2.	Add **250 µl reaction buffer (**REABUF) into each vial (STD, CTRL, sample).
3.	Add **75 µl derivatisation reagent** into each vial (STD, CTRL, sample), and **mix thoroughly** by repeated inversion or several seconds on a vortex mixer. Incubate for **1 hour** at room temperature (15-30 °C) on a **horizontal shaker**.

2 x 50 µl of the derivatised standards/controls/samples are used in the ELISA as duplicates.

Test procedure

Mark the positions of standards/controls/samples in duplicate on a protocol sheet.

Take as many microtiter strips (PLATE) as needed from the kit. Store unused strips covered with foil at 2-8 °C. Strips are stable until expiry date stated on the label.

4.	For the analysis in duplicate take **2 x 50 µl** of the **derivatised standards/ controls/samples** out of the vials and add into the respective wells of the microtiter plate.
5.	Add **50 µl histamine antibody** into each well of the microtiter plate.
6.	Cover the plate tightly with foil and incubate for **1 hour** at room temperature (15-30 °C) on a **horizontal shaker**.
7.	Discard the content of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
8.	Add **100 µl substrate** (SUB) into each well.
9.	Incubate for **12-18 min*** at room temperature (15-30 °C) in the **dark**.
10.	Add **100 µl stop solution** (STOP) into each well and mix well.
11.	Determine **absorption immediately** with an ELISA reader at **450 nm** against 620 nm (or 690 nm) as a reference. If no reference wavelength is available, read only at 450 nm. If the extinction of the highest standard exceeds the range of the photometer, absorption must be measured immediately at **405 nm** against 620 nm (690 nm) as a reference.

* The intensity of the colour change is temperature sensitive. We recommend to observe the colour change and to stop the reaction upon good differentiation.

For automated ELISA processors, the given protocol may need to be adjusted according to the specific features of the respective automated platform. For further details please contact your supplier or Immundiagnostik AG.

8. RESULTS

The following algorithms can be used alternatively to calculate the results. We recommend using the 4 parameter algorithm.

1. **4 parameter algorithm**
   It is recommended to use a linear ordinate for optical density and a logarithmic abscissa for concentration. When using a logarithmic abscissa, the zero standard must be specified with a value less than 1 (e.g. 0.001).
2. **Point-to-point calculation**
   We recommend a linear ordinate for optical density and a linear abscissa for concentration.
3. **Spline algorithm**
   We recommend a linear ordinate for optical density and a linear abscissa for concentration.

The plausibility of the duplicate values should be examined before the automatic evaluation of the results. If this option is not available with the program used, the duplicate values should be evaluated manually.

EDTA plasma

No factor is required for the calculation of results from plasma samples.

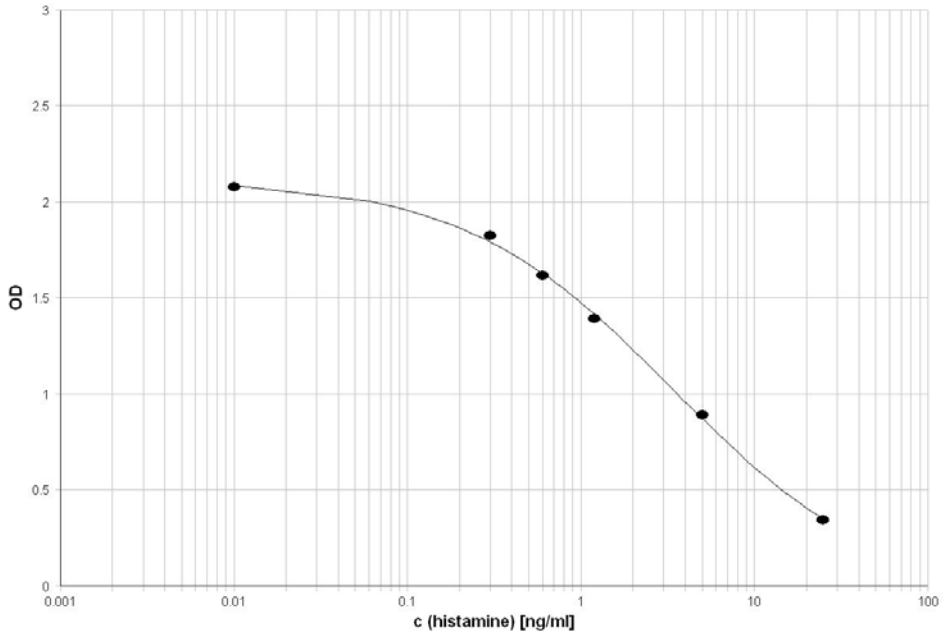
Urine

The obtained histamine levels of urine samples have to be multiplied by the **dilution factor of 15** (ng/ml = µg/l).

Calculation of 24 h excretion: µg/24h = µg/l x l/24h

Conversion: histamine (ng/ml) x 8.997 = histamine (nmol/l)

In the following, an example of a calibration curve is given. Do not use it for the calculation of your results.



9. LIMITATIONS

Samples with concentrations above the measurement range can be diluted with reaction buffer and re-assayed. Please consider this dilution factor when calculating the results.

Samples with concentrations lower than the measurement range cannot be clearly quantified.

The upper limit of the measurement range can be calculated as:

highest concentration of the standard curve × *sample dilution factor to be used*

The lower limit of the measurement range can be calculated as:

analytical sensitivity × *sample dilution factor to be used*

Analytical sensitivity see chapter "Performance Characteristics".

Biotin interference

Samples containing a biotin concentration of < 44 ng/ml show a change of the results of ≤ 25 %. Higher concentrations of biotin can lead to falsely low results. Patients taking > 5 mg biotin per day should wait at least 24 hours after taking biotin to have their samples collected. Results of patients taking biotin supplements or receiving a high-dose biotin therapy should generally be interpreted along with the total clinical picture.

10. QUALITY CONTROL

Immundiagnostik AG recommends the use of external controls for internal quality control, if possible.

Control samples should be analysed with each run. Results generated from the analysis of control samples should be evaluated for acceptability using appropriate statistical methods. The results for the patient samples may not be valid if within the same assay one or more values of the quality control samples are outside of the acceptable limits.

Reference range

Based on internal studies with plasma samples of apparently healthy persons (n = 36) a mean value of 0.67 ng/ml was calculated. The standard deviation (SD) was 0.18 ng/ml. The following normal range was calculated from mean value + 2 SD:

EDTA plasma: < 1 ng/ml

For urine, a mean value of 18.5 µg/g creatinine was calculated based on internal studies with samples of apparently healthy persons (n = 35). The standard deviation (SD) was 10.6. The following normal range was calculated from mean value + 2 SD:

Urine: < 40 µg/g creatinine

We recommend each laboratory to establish its own reference range.

11. PERFORMANCE CHARACTERISTICS

Precision and reproducibility

EDTA plasma

Intra assay (n = 6)

sample	histamine [ng/ml]	CV [%]
1	1.36	8.9
2	3.06	6.2

Inter assay (n = 6)

sample	histamine [ng/ml]	CV [%]
1	0.84	10.0
2	0.54	10.1

Urine

Intra-Assay (n = 6)

sample	histamine [ng/ml]	CV [%]
1	10.10	10.5
2	16.02	8.7

Inter-Assay (n = 6)

sample	histamine [ng/ml]	CV [%]
1	8.49	12.1
2	12.80	8.1

Spiking recovery

Two samples were spiked with different histamine concentrations and measured in this assay. The mean recovery rate was 105.4 % for plasma and 98.9% for urine (n = 2).

EDTA plasma

sample [ng/ml]	spike [ng/ml]	expected [ng/ml]	measured [ng/ml]	recovery [%]
0.96	3	3.96	4.46	112.6
	5	5.96	6.19	103.9
1.01	3	4.01	3.90	97.3
	5	6.01	6.46	107.6

Urine

sample [ng/ml]	spike [ng/ml]	expected [ng/ml]	measured [ng/ml]	recovery [%]
10.10	15	25.10	27.51	109.6
	30	40.10	39.88	99.5
16.03	15	31.03	28.02	90.3
	30	46.03	44.28	96.2

Dilution recovery

Two spiked samples were diluted with reaction buffer. The mean recovery rate was 102.3 % for plasma and 102.4 % for urine (n = 2).

EDTA plasma

sample [ng/ml]	dilution	expected [ng/ml]	measured [ng/ml]	recovery [%]
6.19	1:2	3.09	3.16	102.3
	1:4	1.55	1.70	109.7
6.46	1:2	3.23	2.96	91.6
	1:4	1.62	1.83	113.0

Urine

sample [ng/ml]	dilution	expected [ng/ml]	measured [ng/ml]	recovery [%]
39.88	1:2	19.94	18.64	93.5
	1:4	9.97	9.99	100.2
44.28	1:2	22.14	21.94	99.1
	1:4	11.07	11.70	105.7

Analytical sensitivity

The zero standard was measured 75 times. The detection limit was set as B_0 - 2 SD and estimated to be 0.11 ng/ml. Considering the dilution factor, the detection limit for urine is calculated to be 1.65 ng/ml.

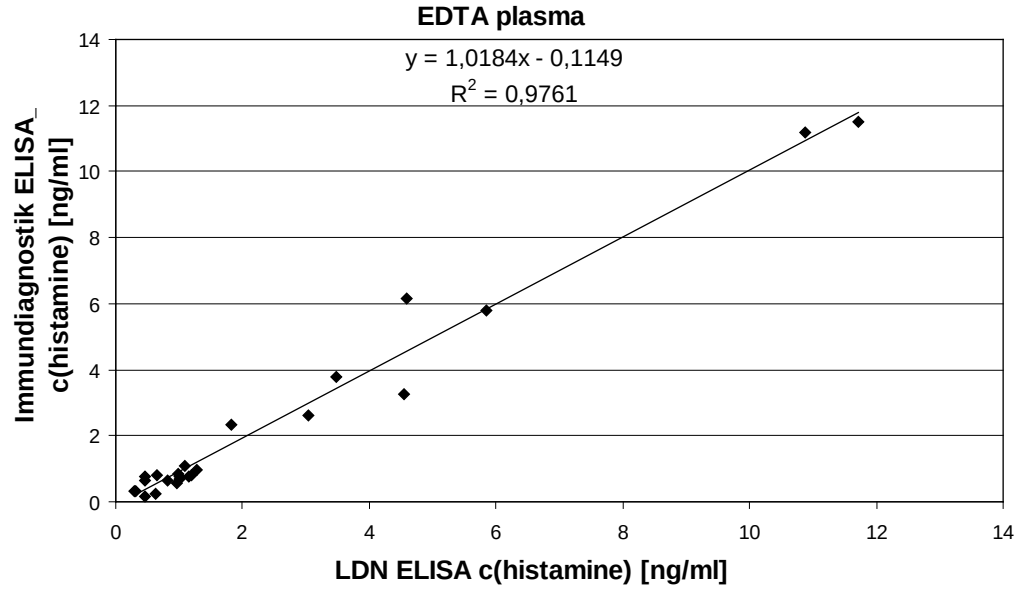
Specificity

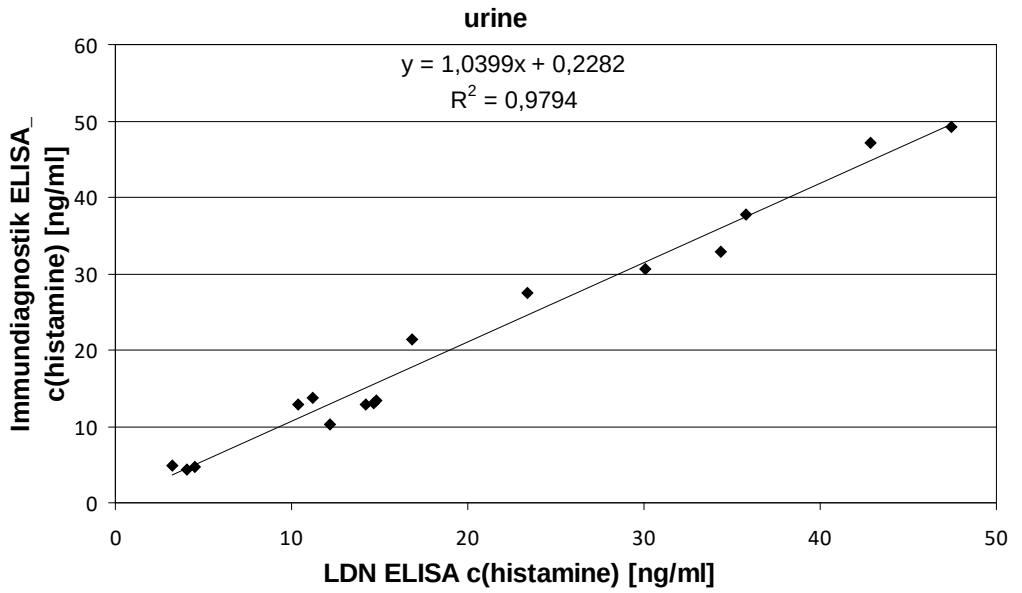
The specificity of the antibody was tested by measuring the cross-reactivity against a range of compounds with structural similarity to histamine. The specificity is calculated in percent in relation to the histamine binding activity:

3-methylhistamine	<0.1 %
tyramine	<0.001 %
L-phenylalanine	<0.0002 %
L-histidine	<0.0002 %
L-tyrosine	<0.0002 %
tryptamine	<0.0002 %
5-hydroxyindoleacetic acid	<0.0002 %
serotonin (5-hydroxytryptamine)	<0.0002 %

Comparison of ELISAs

24 EDTA plasma samples and 16 urine samples were measured with this ELISA and with a commercially available ELISA. The correlation was r = 0.99 for plasma and for urine.





12. PRECAUTIONS

- All reagents in the kit package are for *in vitro* diagnostic use only.
- Kit reagents contain ProClin or thimerosal as bactericides. ProClin and thimerosal are toxic. Substrates for the enzymatic colour reactions are toxic and carcinogenic. Avoid contact with skin or mucous membranes.
- The stop solution consists of diluted sulfuric acid, a strong acid. Although diluted, it still must be handled with care. It can cause burns and should be

handled with gloves, eye protection, and appropriate protective clothing. Any spill should be wiped up immediately with copious quantities of water. Do not breathe vapour and avoid inhalation.

13. TECHNICAL HINTS

- Do not interchange different lot numbers of any kit component within the same assay. Furthermore, we recommend not assembling wells of different microtiter plates for analysis, even if they are of the same batch.
- Control samples should be analysed with each run.
- Reagents should not be used beyond the expiration date stated on the kit label.
- Substrate solution should remain colourless until use.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Avoid foaming when mixing reagents.
- Do not mix plugs and caps from different reagents.
- The assay should always be performed according to the enclosed manual.

14. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- This assay was produced and distributed according to the IVD guidelines of 98/79/EC.
- The guidelines for medical laboratories should be followed.
- Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test. Immundiagnostik AG can therefore not be held responsible for any damage resulting from incorrect use.
- Warranty claims and complaints regarding deficiencies must be logged within 14 days after receipt of the product. The product should be sent to Immundiagnostik AG along with a written complaint.

Used symbols:

 Temperature limitation

REF Catalogue Number

IVD In Vitro Diagnostic Medical Device

→REF To be used with

 Manufacturer

 Contains sufficient for <n> tests

LOT Lot number

 Use by

 Attention

 Consult instructions for use

 Consult specification data sheet