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Manual

For professional use only

Histamine ELISA

For the in vitro determination of histamine in whole blood and dried blood spots

Valid from 2026-07-07

REF **K 8214** Σ 96

REF **K 8214.20** Σ 20 x 96

+2°C +8°C

IVD CE

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Safety information

The assay has to be performed exclusively according to the instructions for use enclosed with the kit.

Important safety information for this product can be found in the chapter WARNINGS AND PRECAUTIONS

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1. INTENDED USE

This Histamine ELISA is an enzyme-linked immunosorbent assay (ELISA) for the quantitative detection of the concentration of total histamine in whole blood and dried blood spots.

The assay is an *in vitro* diagnostic medical device and intended to be used by professional users in a laboratory environment. It can be performed manually or using an automated platform.

The test serves as an aid to diagnosis of an elevated total histamine concentration in blood.

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, increased mucous secretion, tachycardia, alterations of blood pressure and arrhythmias, among other effects. As a vasoactive mediator, it plays a dominant role in allergic diseases such as allergic rhinitis (hay fever), allergic bronchial asthma, and urticaria.

Of clinical interest is the potential histamine release from vesicles in basophilic leukocytes, mast cells or platelets in allergies, which can be estimated by determining the histamine concentration from whole blood or dried blood.

3. MATERIAL SUPPLIED

Cat. No.	Label	Kit Components	Quantity for cat.no.	
			K 8214	K 8214.20
K 8214	PLATE	Microtiter plate, pre-coated	12 x 8 wells	20 x 12 x 8 wells
K 0015	COPLATE	Plate for derivatisation	12 x 8 wells	20 x 12 x 8 wells
K 8214	STD	Standards, ready-to-use (0; 3; 10; 30; 60; 120 ng/ml)	6 x 2 ml	5 x 6 x 2 ml

K 8214	CTRL 1	Control, ready-to-use (see specification for range)	1 x 2 ml	5 x 2 ml
K 8214	CTRL 2	Control, ready-to-use (see specification for range)	1 x 2 ml	5 x 2 ml
K 0001.C.100	WASHBUF	Wash buffer concentrate, 10 x	2 x 100 ml	5 x 100 ml
K 8214	AB	Histamine antibody, peroxidase-labelled, ready-to-use	1 x 6 ml	20 x 6 ml
K 8214	REABUF	Reaction buffer, ready-to-use	2 x 70 ml	30 x 70 ml
K 8214	DER	Derivatisation reagent, lyophilised	1 vials	20 vials
K 0008.07	DMSO	Dimethylsulfoxide (DMSO)	1 x 7 ml	20 x 7 ml
K 0002.15	SUB	Substrate (tetramethylbenzidine), ready-to-use	1 x 15 ml	20 x 15 ml
K 0003.15	STOP	Stop solution, ready-to-use	1 x 15 ml	20 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*
- Dried blood spot carrier such as DrySpot-ID cat. no. DZ9020ID or DZ9021ID
- 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
- Incubator 90 °C
- Centrifuge, 10000 *g*
- 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)** has to be diluted with ultrapure water **1:10** before use (100 ml WASHBUF + 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** can be used until the expiry date stated on the label when stored at **2-8 °C**. **Wash buffer** (1:10 diluted WASHBUF) can be stored in a closed flask at **2-8 °C for 1 month**.
- **DMSO** crystallises at 2-8 °C. Before use, bring to room temperature to melt it.
- The **lyophilised derivatisation reagent (DER)** can be used until the expiry date stated on the label when stored at **2-8 °C**. Bring to room temperature before opening to prevent condensation. 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. Ensure that it is completely dissolved. **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 plastics but not polypropylene products and laboratory glass.
- All other test reagents are ready-to-use. Test reagents can be used until the expiry date stated on the label when stored at **2-8 °C.**

6. STORAGE AND PREPARATION OF SAMPLES

Whole blood

Sample storage

Heparinised whole blood is suitable as sample material. In heparinised whole blood, histamine is stable for 3 days at room temperature or at 2-8 °C. For longer storage keep samples frozen at -20 °C.

Extraction

1.	Add **50 µl heparinised whole blood sample** in labelled 1.5 ml polypropylene tubes.
2.	Add **1 ml reaction buffer** (REABUF) to each sample and mix well.

3.	Heat the samples for **10 minutes at 90°C**.
4.	Then let cool for **10 minutes**.
5.	Cetrifuge for **5 minutes** at **10000 *g***.

To **275 µl** of this extract a derivatisation reagent is added (see derivatisation procedure).

Dried blood spots

Collection and storage of dried blood spots

50 µl whole blood dripped on a dried sample carrier cleared by Immundiagnostik AG are suitable as sample material after complete drying. We recommend DrySpot-ID (catalogue no. DZ9020ID or DZ9021ID) as dried blood spot carrier. The moistened cards are stable for 7 days at room temperature. For longer storage, store at -20°C in a dry place.

Extraction

1.	Remove filter from sampling device and put it in a labelled 1.5 ml poly-propylene tube.
2.	Add **1 ml reaction buffer** (REABUF) to each sample and mix well.
3.	Heat the samples for **10 minutes at 90°C**.
4.	Then let cool for **10 minutes**.
5.	Cetrifuge for **5 minutes** at **10000 *g***.

To **275 µl** of this extract a derivatisation reagent is added (see derivatisation procedure).

7. ASSAY PROCEDURE

Principle of the test

This ELISA is designed for the quantitative determination of histamine in heparinised whole blood and dried bood spots. 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, immobilised 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.

Derivatisation procedure

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

Derivatisation of standards, controls and extracted samples is carried out in reaction vials (e.g. 1.5 ml polypropylene vials). Alternatively, the derivatisation can be carried out in the wells of the COPLATE.

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 **25 µl standard** (STD)/**control** (CTRL) into the respective vials, or into the wells of the COPLATE. Add **275 µl extracted sample** into the respective vials, or into the wells of the COPLATE.
2.	Add **250 µl reaction buffer** (REABUF) **only to the standards and controls** in the vials or the wells of the COPLATE.
3.	Add **50 µl derivatisation reagent** into each vial (STD, CTRL, sample) and **mix thoroughly** by repeated inversion or several seconds on a vortex mixer. Incubate for **30 min** at room temperature (15-30 °C) on a **horizontal shaker**. *Alternatively:* Add **50 µl derivatisation reagent** into each well (STD, CTRL, sample) of the COPLATE and incubate immediately on a **horizontal shaker** for **30 min** at room temperature (15-30 °C).

2 x 50 µl of the derivatised standards, controls and 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 together with the desiccant bag in the closed aluminium packaging at 2-8 °C. Strips are stable until the 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, or the COPLATE, and add into the respective wells of the microtiter plate.
5.	Add **50 µl histamine antibody** (AB) into each well of the microtiter plate.
6.	Cover the strips 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. Attention should be paid to the following points:

Derivatisation of the samples is possible in 30 minutes on a horizontal shaker or, after thorough mixing, in 90 minutes without shaking in an automated processor. In automated processing, the volumes of samples and diluents may be scaled while

maintaining the respective dilutions. Device-specific minimum and maximum volumes must be taken into account. The homogeneity of the resulting dilution must be ensured.

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.

Whole blood and dried blood spots

The obtained results have to be multiplied by the **dilution factor of 1.91** to get the actual concentrations.

9. LIMITATIONS

Samples with concentrations above the measurement range cannot be diluted.

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 ≤ 129 ng/ml show a change of the results of < 25 %. Higher concentrations of biotin can lead to false 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 in-house studies with whole blood and dried blood samples of apparently healthy persons (n = 38), a median of 39 ng/ml was determined for whole blood, the 10^{th} and 90^{th} percentile were 26 and 63 ng/ml, respectively. For dried blood, a median of 46 ng/ml was determined, the 10^{th} and 90^{th} percentile were 29 and 72 ng/ml, respectively. This results in the following normal ranges:

Histamine concentration in whole blood: 26 – 63 ng/ml

Histamine concentration in dried blood spots: 29 – 72 ng/ml

We recommend each laboratory to establish its own reference range.

11. PERFORMANCE CHARACTERISTICS

Precision and reproducibility

Repeatability (Intra-Assay); n = 12

The repeatability was assessed with 4 samples under constant parameters (same operator, measurement system, day and kit lot) in single determinations.

Whole blood

Sample	Mean value [ng/ml]	CV [%]
1	30.3	9.1
2	52.6	8.8
3	74.6	9.4
4	87.4	5.4

Dried blood spot

Sample	Mean value [ng/ml]	CV [%]
1	37.2	7.1
2	51.2	8.0
3	65.0	7.9
4	93.7	9.7

Reproducibility (Inter-Assay); n = 12

The reproducibility was assessed with 6 samples under varying parameters (different operators, measurement systems, days and kit lots) in duplicate determinations.

Whole blood

Sample	Mean value [ng/ml]	CV [%]
1	26.8	12.4
2	31.7	13.9
3	42.4	9.5
4	59.9	7.4
5	83.8	7.9
6	173.1	11.3

Dried blood spot

Sample	Mean value [ng/ml]	CV [%]
1	27.9	16.8
2	34.4	8.5
3	48.1	7.6
4	56.2	12.0
5	94.2	7.5
6	176.6	11.6

Accuracy – Trueness

The trueness states the closeness of the agreement between the result of a measurement and the true value of the measurand. Therefore, histamine spikes with known concentrations were added to 3 different samples.

Whole blood

sample [ng/ml]	spike [ng/ml]	expected [ng/ml]	obtained [ng/ml]	recovery [%]
30.9	16.9	47.8	47.0	98.2
	36.9	67.8	69.0	101.6
	56.9	87.8	90.3	102.8
32.9	18.6	49.6	47.4	95.6
	38.6	69.6	66.1	95.0
	58.6	89.6	109.3	122.0
30.0	16.0	47.0	42.6	90.6
	36.0	67.0	62.3	93.0
	56.0	87.0	91.7	105.4

Dried blood spot

sample [ng/ml]	spike [ng/ml]	expected [ng/ml]	obtained [ng/ml]	recovery [%]
27.4	17.3	44.6	40.9	91.6
	37.3	64.6	59.3	91.7
	57.3	84.6	98.3	116.2
28.4	18.2	45.6	44.2	96.9
	38.2	65.6	54.8	83.6
	58.2	85.6	85.3	99.7
30.0	19.7	47.0	46.0	97.7
	39.7	67.0	56.9	84.9
	59.7	87.0	94.6	108.6

Linearity

The linearity states the ability of a method to provide results proportional to the concentration of analyte in the test sample within a given range. This was assessed with a serial dilution of 4 heparinised whole blood samples with a Li-heparin plasma sample containing 2.39 ng/ml histamine.

For histamine in heparinised whole blood, the method has been demonstrated to be linear from 11.9 to 118.0 ng/ml with a recovery rate of 82.8 to113.4 %.

For histamine in dried blood spots, the method has been demonstrated to be linear from 14.2 to 121.6 ng/ml with a recovery rate of 79.7 to 106.4 %.

Whole blood

sample [ng/ml]	dilution	expected [ng/ml]	obtained [ng/ml]	recovery [%]
40.6	1:1.5	27.9	23.7	85.2
	1:2	21.5	18.2	84.9
	1:2.5	17.7	14.6	82.9
	1:3	15.1	12.5	82.8
	1:4	11.9	12.4	103.9
56.2	1:1.5	38.3	37.4	97.8
	1:2	29.3	30.2	103.1
	1:2.5	23.9	26.5	110.9
	1:3	20.3	21.3	104.8
	1:4	15.9	15.0	94.8
98.9	1:1.5	66.7	59.2	88.8
	1:2	50.6	44.0	86.9
	1:2.5	41.0	35.4	86.3
	1:3	34.5	33.4	96.6
	1:4	26.5	27.7	104.5
118.0	1:1.5	79.5	82.4	103.7
	1:2	60.2	58.9	97.9
	1:2.5	48.6	50.0	102.9
	1:3	40.9	42.0	102.7
	1:4	31.3	35.5	113.4

Dried blood spot

sample [ng/ml]	dilution	expected [ng/ml]	obtained [ng/ml]	recovery [%]
39.9	1:1.5	27.4	24.7	90.1
	1:2	21.2	19.2	90.8
	1:2.5	17.4	15.5	89.0
	1:3	14.9	13.5	90.7
	1:4	11.8	8.9	75.8
49.7	1:1.5	33.9	33.3	98.1
	1:2	26.0	27.3	104.7
	1:2.5	21.3	21.2	99.5
	1:3	18.2	19.3	106.4
	1:4	14.2	13.3	93.9

78.9	1:1.5	53.4	51.7	96.7
	1:2	40.7	44.1	108.4
	1:2.5	33.0	26.3	79.7
	1:3	27.9	23.2	83.1
	1:4	21.5	22.0	102.0
121.6	1:1.5	81.9	68.0	83.1
	1:2	62.0	50.3	81.1
	1:2.5	50.1	40.7	81.4
	1:3	42.1	34.3	81.5
	1:4	32.2	28.9	89.8

Analytical sensitivity

The following values have been estimated based on the concentrations of the standard curve without considering possibly used sample dilution factors.

Whole blood

The zero-standard was measured 40 times. The detection limit was set as B_0 - 2 SD and estimated to be 1.18 ng/ml.

Dried blood spot

The zero-standard was extracted from a dried sample carrier and measured 40 times. The detection limit was set as B_0 - 2 SD and estimated to be 2.01 ng/ml.

Analytical Specificity

The pecificity 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 %

12. WARNINGS AND 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 harmful to health and the environment. Substrates for enzymatic colour reactions can also cause skin and/or respiratory irritation. Any contact with the substances should be avoided. Further safety information can be found in the safety data sheet, which is available from Immundiagnostik AG on request.
- The 10x Wash buffer concentrate (WASHBUF) contains surfactants which may cause severe eye irritation in case of eye contact.

  Warning: Causes serious eye irritation. **IF IN EYES:** Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. If eye irritation persists: Get medical advice/attention.
- 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.

15. REFERENCES

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16. SYMBOLS


Temperature limitation

In Vitro Diagnostic Medical Device


Manufacturer

Lot number


Contains plasma derivates or human blood

Consult product specification data sheet


European Conformity

Unique Device Identification


Medical substance

Catalogue number

To be used with

Contains sufficient for <n> tests


Use by


Consult instructions for use


Do not re-use

Contains material of animal origin

Contains material of human origin


Irritant