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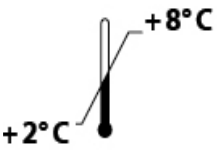
Manual

Histamine elimination ratio (HERO) ELISA

For the in vitro determination of histamine elimination in serum

Valid from 2022-05-23

REF **K 8215**



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1. INTENDED USE

The histamine assay K 8215 is an enzyme-linked immunosorbent assay (ELISA) intended for professional laboratory users for the quantitative measurement of serum histamine elimination from patients of any age and gender.

The assay is an *in vitro* diagnostic medical device, which can be used manually or by an automated platform.

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.

Histamine is mainly degraded by two pathways: By the extracellular diamonioxidase (DAO) and by the predominantly intracellular histamine N-methyltransferase (HNMT).

Measurement of serum histamine depletion is of clinical interest in patients showing one of the above-mentioned allergic or pseudoallergic reactions to histamine-rich foods.

3. MATERIAL SUPPLIED

Cat. No.	Label	Kit Components	Quantity
K 8215	PLATE	Microtiter plate, pre-coated	2 x 12 x 8 wells
K 0015	COPLATE	Plate for derivatisation	2 x 12 x 8 wells
K 8215	INCPLATE	Plate for sample incubation	12 x 8 wells
K 8215	STD	Standards, ready-to-use (0; 1; 3; 10; 30; 120 ng/ml)	6 x 2 ml
K 8215	CTRL 1	Control, ready-to-use (see specification for range)	1 x 2 ml

K 8215	CTRL 2	Control, ready-to-use (see specification for range)	1 x 2 ml
K 0001.C.100	WASHBUF	Wash buffer concentrate, 10 x	2 x 100 ml
K 8215	SPIKEREAG	Spiking reagent containing histamine	1 x 22 ml
K 8215	AB	Histamine antibody, peroxidase-labelled, ready-to-use	2 x 6 ml
K 8215	REABUF	Reaction buffer, ready-to-use	1 x 70 ml
K 0008.10	DER	Derivatisation reagent, lyophilised	2 vials
K 0002.15	DMSO	Dimethylsulfoxide (DMSO)	2 x 10 ml
K 0003.15	SUB	Substrate (tetramethylbenzidine), ready-to-use	2 x 15 ml
K 8215	STOP	Stop solution, ready-to-use	2 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
- Incubator 37 °C
- 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 dissolve the crystals.
- 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 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 can be used until the expiry date stated on the label when stored at **2-8 °C.**

6. STORAGE AND PREPARATION OF SAMPLES

Serum samples are stable for 3 days at room temperature or for 14 days at 2-8 °C. For longer storage keep samples frozen at -20 °C.

Serum samples are analysed **undiluted**.

For sample preparation, samples are spiked with histamine and incubated, and a derivatisation reagent is added (see assay procedure).

7. ASSAY PROCEDURE

Principle of the test

This ELISA is designed for the quantitative determination of histamine elimination in serum.

The assay consists of two steps: spiking of the serum samples with a histamine-containing reagent and incubation, and determination of histamine concentrations in the ELISA.

Incubation of samples

A histamine-containing spiking reagent is added to the serum sample and it is incubated at 37 °C for 24 hours. The histamine concentration of this sample is then determined by ELISA.

A reference sample is prepared by storing an aliqout of the serum sample at 2-8 °C for 24 hours and then adding the spiking reagent immediately before determining the histamine concentration in the ELISA.

Determination of histamine concentrations in the ELISA

This assay is based on the method of competitive enzyme linked immunoassays.

The preparation of standards, controls, incubated samples and reference samples 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.

Incubation of samples

Take two aliquots of the serum samples, for incubated sample and for reference sample:

	Incubated samples:	**Reference samples:**
1.	Add **100 µl** serum into the incubation plate (INCPLATE) or in 1,5 ml poly-propylene reaction vials.	store **125 µl** serum for **24 hours at 2-8 °C** as a reference.
2.	Add **200 µl** spiking reagent (SPIKEREAG) to each sample in the incubation plate or the vials. **Close** the incubation plate **tightly** with the cap mat or close the vials, respectively. Mix thoroughly on a **horizontal shaker** for **1 minute**.	

3.	Incubate for **24 hours at 37 °C**.	
4.		The next day: Add **100 µl** of the reference sample into the incubation plate (INCPLATE) or in polypropylene reaction vials.
5.		Add **200 µl** spiking reagent (SPIKEREAG) to each reference sample and mix thoroughly on a **horizontal shaker** for **1 minute**.

Then **immediately** process the reference samples and the incubated samples together, see derivatisation procedure.

Derivatisation procedure

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

Derivatisation of standards and controls, incubated samples and reference 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 (PLATE).

6.	Add **25 µl standard** (STD)/**control** (CTRL)/ **incubated sample**/ **reference sample** into the respective vials, or into the wells of the COPLATE.
7.	Add **250 µl reaction buffer** (REABUF) into the vials (STD, CTRL, samples) or the wells of the COPLATE.
8.	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). Cover the COPLATE with the supplied lid, do not cover it with foil.

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

Test procedure

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.

9.	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.
10.	Add **50 µl histamine antibody** (AB) into each well of the microtiter plate.
11.	Cover the strips tightly with foil and incubate for **1 hour** at 18-25 °C on a **horizontal shaker**.
12.	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.
13.	Add **100 µl substrate** (SUB) into each well.
14.	Incubate for **12-18 min*** at 18-25 °C in the **dark**.
15.	Add **100 µl stop solution** (STOP) into each well and mix well.
16.	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.

Calculation of the histamine elimination ratio

For the calculation of the histamine elimination ratio (HERO), the difference of the histamine concentrations of reference and incubated sample is divided by the concentration of the reference sample.

$$\text{HERO [\%]} = \frac{c\,(histamine)\;reference - c\,(histamine)\;incubated\;sample}{c\,(histamine)\;reference}\;x\;100$$

9. LIMITATIONS

Biotin interference

Samples containing a biotin concentration of ≤ 343 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 internal studies with samples of apparently healthy persons (n = 42) a mean histamine elimination ratio of 45 % (median) was calculated. Weak, moderate and good elimination was defined as follows:

Weak Elimination:	HERO	< 25 %
Moderate Elimination:	HERO	25-40 %
Good Elimination	HERO	> 40 %

We recommend each laboratory to establish its own reference range.

11. PERFORMANCE CHARACTERISTICS

Precision and reproducibility

Repeatability (Intra-Assay); n = 6

The repeatability was assessed with 2 samples under **constant** parameters (same operator, measurement system, day and kit lot) in single determinations.

Sample	Mean value incubated sample [%]	Mean value reference sample [%]	Mean value HERO [%]	CV [%]
1	1.8	23.0	92.2	3.4
2	8.2	20.7	60.2	6.3

Reproducibility (Inter-Assay); n = 6

The reproducibility was assessed with 6 samples from 2 persons under **varying** parameters (different operators, measurement systems, days and kit lots) in duplicate determinations.

Samples from	Mean value incubated sample [%]	Mean value reference sample [%]	Mean value HERO [%]	CV [%]
A	2.8	18.1	84.4	8.0
B	7.5	18.1	57.8	11.1

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.

sample [ng/ml]	spike [ng/ml]	expected [ng/ml]	obtained [ng/ml]	recovery [%]
0.35	3.10	3.45	3.61	104.7
	6.43	6.78	6.35	93.7
	9.77	10.12	8.65	85.5
0.49	3.01	3.50	3.48	99.6
	6.34	6.83	6.27	91.8
	9.68	10.16	8.67	85.4
0.59	2.94	3.53	3.36	95.1
	6.28	6.86	5.94	86.6
	9.61	10.20	8.02	78.6

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 spiked serum samples with a low level serum sample containing 0.6 ng/l histamine.

For histamine in serum, the method has been demonstrated to be linear from 3.0 to 30.5 ng/ml with a recovery rate of 83.6 to117.9 %.

sample [ng/ml]	dilution	expected [ng/ml]	obtained [ng/ml]	recovery [%]
19.8	1:1.5	13.4	13.7	102.6
	1:2	10.2	10.5	103.5
	1:3	7.0	7.1	101.4
	1:4	5.4	5.6	104.2
	1:5	4.4	5.2	117.9
	1:6	3.8	4.0	106.2
	1:8	3.0	2.8	93.8

23.5	1:1.5	15.8	14.9	94.3
	1:2	12.0	10.4	86.1
	1:3	8.2	7.5	91.6
	1:4	6.3	6.1	97.5
	1:5	5.2	4.4	84.3
	1:6	4.4	4.4	99.9
	1:8	3.4	3.3	95.8
30.5	1:1.5	20.5	20.4	99.4
	1:2	15.5	13.0	83.6
	1:3	10.6	9.8	93.2
	1:4	8.1	8.5	104.9
	1:5	6.6	6.9	105.4
	1:6	5.6	6.3	113.3
	1:8	4.3	5.1	118.6
22.8	1:1.5	15.4	16.1	104.4
	1:2	11.7	10.1	86.2
	1:3	8.0	8.9	111.7
	1:4	6.1	5.3	86.2
	1:5	5.0	4.4	86.5
	1:6	4.3	4.0	93.2
	1:8	3.4	3.4	99.6

Analytical sensitivity

The matrix of the spiking reagent was measured 80 times. The detection limit was set as B_0 - 2 SD and estimated to be 0.9 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 %

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 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 ELISA 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

1. Küfner MA, Schwelberger HG, Ulrich P, Hahn EG, Raithel M. Total histamine degradation capacity (THDC) as an important biological marker of histamine metabolism in human colonic mucosa. *Inflamm Res.* 2002 Apr;51 Suppl 1:S87-8. doi: 10.1007/pl00022461. PMID: 12013425
2. Küfner MA, Ulrich P, Raithel M, Schwelberger HG. Determination of histamine degradation capacity in extremely small human colon samples. *Inflamm Res.* 2001 Apr; 50 Suppl 2:S96-7. doi: 10.1007/PL00022422. PMID: 11411621.
3. Raithel M, Küfner MA, Ulrich P, Hahn EG. The involvement of the histamine degradation pathway by diamine oxidase in manifest gastrointestinal allergies. *Inflamm Res.* 1999 Apr; 48 Suppl 1: S75-6. doi: 10.1007/s000110050414. PMID: 10350171.

Used symbols:

	Temperature limitation		Catalogue number
	In Vitro Diagnostic Medical Device		To be used with
	Manufacturer		Contains sufficient for <n> tests
	Lot number		Use by
	Contains plasma derivatives or human blood		Consult instructions for use
	Consult specification data sheet		Do not re-use
	Unique Device Identification		Contains material of animal origin
	Medicinal substance		Contains material of human origin