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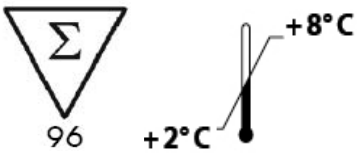
Manual

Histamine ELISA

For the in vitro determination of histamine in stool

Valid from 2020-09-01

REF **K 8213**



IVD CE

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1. INTENDED USE

This Immundiagnostik AG assay is intended for the quantitative determination of histamine in stool. 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 8213	PLATE	Microtiter plate, pre-coated	12 x 8 wells
K 8213	COPLATE	Plate for derivatisation	12 x 8 wells
K 8213	STD	Standards, ready-to-use (0; 1; 3; 10; 30; 120 ng/ml)	6 x 2 ml
K 8213	CTRL 1	Control, ready-to-use (see specification for range)	1 x 2 ml
K 8213	CTRL 2	Control, ready-to-use (see specification for range)	1 x 2 ml
K 0006.C.100	WASHBUF A	Wash buffer concentrate, 10 x	2 x 100 ml
K 7999.100	IDK® Amino Extract	Extraction buffer, ready-to-use	2 x 100 ml
K 8213	AB	Histamine antibody, peroxidase-labelled, ready-to-use	1 x 6 ml
K 8213	REABUF	Reaction buffer, ready-to-use	1 x 30 ml

K 8213	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*
- Stool sample preparation system such as Cat. No: K 7999
- 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
- 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, bring to room temperature to dissolve the crystals.
- 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

Extraction of the stool samples

We recommend using the **stool sample preparation system filled with IDK® Amino extract** (extraction buffer), **Cat No. K 7999**.

The extraction buffer IDK® Amino Extract is ready-to-use. We recommend the following sample preparation:

Stool sample tube – Instructions for use

Please note that the dilution factor of the final stool suspension depends on the amount of stool sample used and the volume of the buffer:

Stool sample tube with 0.75 ml buffer:

Applied amount of stool:	15 mg
Buffer volume (IDK® Amino Extract):	0.75 ml
Dilution factor:	1:50

Please follow the instructions for the preparation of stool samples using the stool sample tubes as follows:

a) The raw stool sample has to be thawed. For particularly heterogeneous samples we recommend a mechanical homogenization using an applicator, inoculation loop or similar device.

b) Unscrew the tube (yellow part of cap) to open. Insert the yellow dipstick into the sample. The lower part of the dipstick has notches which need to be covered completely with stool after inserting it into the sample. Place the dipstick back into the tube. When putting the stick back into the tube, excess material will be stripped of, leaving 15 mg of sample to be diluted. Screw tightly to close the tube.

c) Shake the tube well until no stool sample remains in the notches. Important: Please make sure that you have a maximally homogenous suspension after

shaking. Especially with more solid samples, soaking the sample in the tube with buffer for ~10 minutes improves the result.

d) Allow sample to stand for ~10 minutes until sediment has settled. Floating material like shells of grains can be neglected.

f) Carefully unscrew the complete cap of the tube including the blue ring plus the dipstick. Discard cap and dipstick. Make sure that the sediment will not be dispersed again.

Dilution I: 1:50

Dilution of samples

Dilute the supernatant of the sample extraction (dilution I) **1:4 with extraction buffer (IDK® Amino Extract).** For example:

100 µl supernatant (dilution I) + **300 µl IDK® Amino Extract**, mix well = **1:4** (**dilution II**). This results in a final dilution of **1:200**.

To **25 µl of dilution II** a derivatisation reagent is added for derivatisation of histamine (see derivatisation procedure).

Sample storage

Raw stool is stable for 2 days at room temperature. For longer storage keep frozen at -20 °C.

Stool extract is stable for 2 days at room temperature. For longer storage keep frozen at -20 °C.

7. ASSAY PROCEDURE

Principle of the test

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

Derivatisation procedure

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

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

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)**/sample from dilution II** into the respective wells of the COPLATE or into the vials.
2.	Add **250 µl reaction buffer** (REABUF) into each well of the COPLATE or into each vial (STD, CTRL, sample).
3.	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). *Alternatively:* 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**.

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 COPLATE, or 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 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. 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.

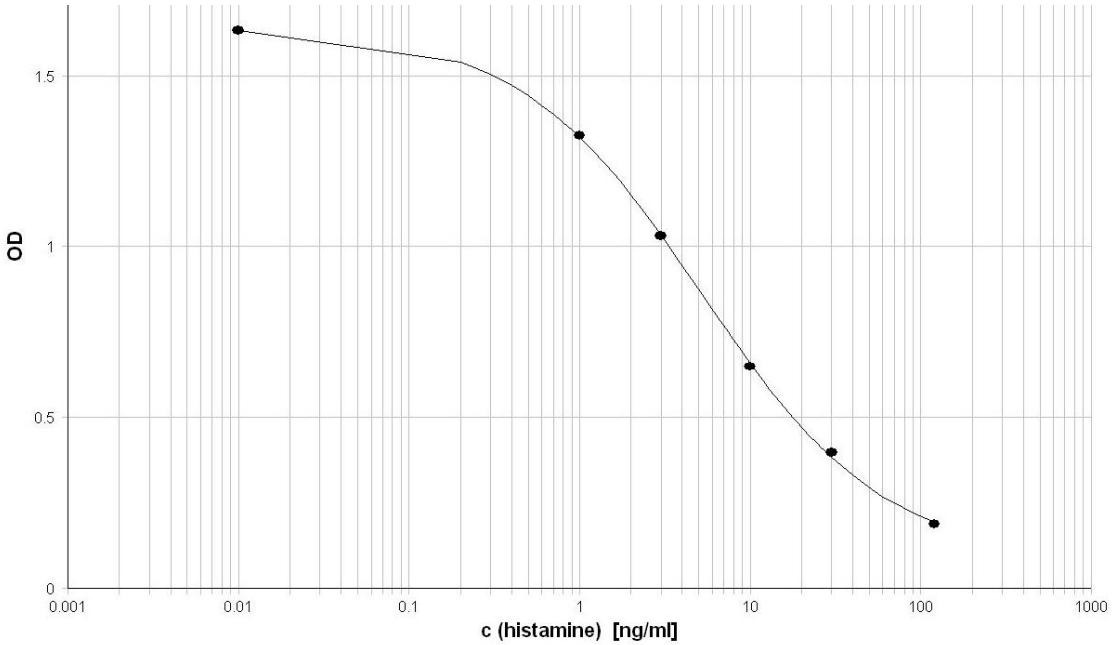
The plausibility of the duplicate values should be examined before the automatic evaluation of the results. If this option is not available with the program used, the duplicate values should be evaluated manually.

Stool samples

The obtained histamine levels of the stool samples have to be multiplied by the **dilution factor of 200** (1 ng/ml = 1 ng/g stool).

In case another dilution factor has been used, multiply the obtained result by the dilution factor used.

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 extraction buffer (IDK® Amino Extract) and re-assayed. Please consider this higher dilution 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:

LoB × sample dilution factor to be used

LoB see chapter "Performance Characteristics".

Biotin interference

Samples containing a biotin concentration of < 1333 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 stool samples of apparently healthy persons (n = 36) a mean value of 481 ng/ml was calculated. The standard deviation (SD) was 239 ng/ml. The following normal range was calculated from mean value + 2 x SD:

Stool: < 959 ng/ml (ng/g stool)

We recommend each laboratory to establish its own reference range.

11. PERFORMANCE CHARACTERISTICS

Precision and reproducibility

Intra-assay (n = 12)

sample	histamine [ng/ml]	CV [%]
1	689	8.8
2	1331	8.3

Inter-assay (n = 10)

sample	histamine [ng/ml]	CV [%]
1	361	7.7
2	1502	8.8

Spiking recovery

Two samples were spiked with different histamine concentrations and measured in this assay. The mean recovery rate was 98.2 % (n = 2).

sample	spike [ng/ml]	expected [ng/ml]	measured [ng/ml]	recovery [%]
A			175.0	
	400	575.0	555.0	96.5
	1320	1495.0	1579.0	105.6
B			232.0	
	400	632.0	583.0	92.2
	1320	1552.0	1530.0	98.6

Dilution recovery

Two spiked samples were diluted and measured in this assay. The mean recovery rate was 91.8 % (n = 2).

sample	dilution	expected [ng/ml]	measured [ng/ml]	recovery [%]
A	1:200		2157.4	
	1:400	1078.7	987.6	91.6
	1:800	539.4	468.4	86.8
	1:1600	269.7	261.8	97.1
B	1:200		3155.4	
	1:400	1577.7	1647.6	104.4
	1:800	788.9	718.4	91.1
	1:1600	394.4	327.3	83.0

Analytical sensitivity

The following values have been estimated based on the standard curve in consideration of the sample dilution factor of 200.

Limit of blank, LoB	99 ng/ml
Limit of detection, LoD	154 ng/ml
Limit of quantitation, LoQ	200 ng/ml

The evaluation was performed according to the CLSI guideline EP-17-A2. The specified accuracy goal for the LoQ was 20 % CV.

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. 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

Catalogue Number

In Vitro Diagnostic Medical Device

To be used with


Manufacturer

Contains sufficient for <n> tests

Lot number


Use by


Attention


Consult instructions for use

Consult specification data sheet