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Instructions for Use

1-Methylhistamine ELISA

Enzyme Immunoassay for the quantitative Determination of 1-Methylhistamine (N-Methylhistamine) in Urine

REF **RE59231**

Σ **96**

2°C 8°C

For illustrative purposes only.
To perform the assay the instructions for use provided with the kit have to be used.

Distributed by: **IBL International GmbH**
Flughafenstrasse 52a
22335 Hamburg, Germany

Always there for you

Instruction for use

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Enzyme Immunoassay
for the quantitative Determination of
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CE

IVD

REF RE59231 (MHE00)

Σ 96

+8 °C +2 2 – 8 °C

Version 7, Feb. 2026

1. Introduction and Principle of the Test

Histamine (β-imidazole-ethylamine) a biogenic amine, is a product of the histidine metabolism. It is produced by decarboxylation of histidine.

Histamine is widely distributed in mammalian tissues. It's bound to heparin (as inactive form) and stored in the granules of basophilic leukocytes and mast cells and is actively released as required. These cells, if sensitized by IgE antibodies attached to their membranes, degranulate when exposed to the appropriate antigen.
Histamine plays a major rule in the initial phase of an anaphylactic reaction.
The quantification of histamine in plasma after allergen administration is of clinical interest.

Histamine is part of the immune response to foreign pathogens and it increases the permeability of the capillaries to white blood cells and other proteins, in order to allow them to engage foreign invaders in the affected tissues.

1-Methylhistamine is a histamine metabolite. It is a product of histamine 1- methyltransferase. Urinary histamine and 1-methylhistamine are highly correlated with histamine in plasma. Therefore, allergic reactions can be examined by determination of histamine and 1-methylhistamine in urine.

The assay kit provides materials for the quantitative measurement of derivated 1-methylhistamine in human urine. The derivation is performed by using the acylation reagent. The 1-methylhistamine is quantitatively derivated into N-acylmethylhistamine.

The competitive 1-Methylhistamine ELISA kit uses the microtitre plate format. 1-methylhistamine is bound to the solid phase of the microtiter plate. Acylated 1-methylhistamine and solid phase bound 1-methylhistamine compete for a fixed number of antiserum binding sites. When the system is in equilibrium, free antigen and free antigen-antiserum complexes are removed by washing. The antibody bound to the solid phase antigen is detected by anti-rabbit/peroxidase. The substrate TMB / peroxidase reaction is monitored at 450 nm. The amount of antibody bound to the solid phase antigen is inversely proportional to the 1-methylhistamine concentration of the sample.

2. Precautions

- For in-vitro diagnostic use only. For professional use only.
- Before carrying out the test, the valid instruction for use, as included in this kit, should be read completely and the content understood.
- Material of animal origin used in the preparation of the kit has been obtained from animals certified as healthy but these materials should be handled as potentially infectious.
- Obey lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.
- By handling reagents, controls and samples follow good laboratory practice and safety guidelines.
- Wear lab coats, disposable gloves and protective glasses.
- Some of the components of this test kit are subject to labeling. These components bear the corresponding hazard symbol on their label. For further information, please see Chapter 4 Contents of the Kit and the relevant safety data sheets.
- Avoid any actions that could result in ingestion, inhalation or injection of the reagents. Never pipette by mouth.
- Avoid contact with reagents.
- Dispose waste according to state and local environmental protection regulation.
- Some components contain small amounts of sodium azide as a preservative. Prevent the formation of heavy metal azides in the drain system by flushing with copious amounts of water.
- Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled samples.

3. Storage and Stability

The kit is shipped at ambient temperature. On arrival, store the kit at 2 - 8 °C to keep it stable until its expiry date. Once opened the kit is stable until its expiry date. The shell life of the ready to use reagents is indicated on the respective bottle label. For stability of prepared reagents refer to 6. Preparation of Reagents.
Allow all reagents to reach room temperature before use and refrigerate them immediately after use.

4. Contents of the Kit

4.1 **Microtiter Strips** **STRIPS** 12 strips
8 wells each, able to break off
precoated with N-acyl- 1-methylhistamine

4.2 **Standards 1 - 6** **CAL 1 - 6** 6 vials
Each 4 ml, ready for use
Concentrations:

Standard:		1	2	3	4	5	6
1-Methylhistamine	ng / ml	0	10	30	100	300	1000
	nmol / l	0	80	240	800	2400	8000

4.3 **Control 1 & 2** **CON 1 & 2** 2 vials
Each 4 ml, ready for use
Range: see q.c. certificate

4.4 **Acylation Reagent** **ACYL-REAG** 3 vials
lyophilised, dissolve content
in 3 ml SOLVENT , if necessary
combine the contents of two vials (see 6. also)

4.5 **Solvent** **SOLVENT** 1 vial
11 ml, ready for use, colour coded yellow
Solvent to dissolve the Acylation reagent

4.6 **Antiserum** **AS**  Warning 1 vial
6 ml, ready for use, colour coded yellow
Rabbit-anti-N-acyl-1-methylhistamine

4.7 **Enzyme Conjugate** **CONJ**  Warning 1 vial
13 ml, ready for use
Goat anti-rabbit-IgG-peroxidase

4.8 **Wash Buffer** **WASH** 1 vial
20 ml, concentrated (50x)

4.9 **Substrate** **SUB** 1 vial
13 ml TMB solution, ready for use

4.10 **Stop Solution** **STOP** 1 vial
13 ml, ready for use
contains 0.3 M sulphuric acid

4.11 **Preparation plate** **PRE-PLATE** 2 pieces
for sample preparation

4.12 **Equalizing Reagent** **EQUA-REAG** 1 vial
lyophilized, dissolve content with 32 ml ACYL-BUFF

4.13 **Acylation Buffer** **ACYL-BUFF** 1 vial
32 ml, ready for use, colour coded blue
To dissolve the Equalizing reagent

Additional materials and equipment required but not provided:

- Pipettes (20, 50, 100, 300 µl) and Multipette
- Orbital shaker
- Multichannel pipette or Microplate washing device
- Microplate photometer (450 nm)
- Distilled water
- Vortex mixer and roller mixer
- Paper towels, pipette tips, timer
- Centrifuge

5. Sample Collection

Urine

Spontaneous urine can be used for this test as well as collected urine. In this case the total volume of urine excreted during a 24-hours period should be collected and mixed in a single bottle containing 10 - 15 ml of 6 M hydrochloric acid (Warning: Observe hazard warning) as preservative. Avoid exposure to direct sun light. Determine the total volume and take an aliquot for the measurement. For patients with suspected kidney disorders the creatinine concentration should be tested, too. Urine samples can be stored at -20 °C for at least 6 months.

Repeated freezing and thawing should be avoided.

Mix and centrifuge urine before use.

6. Preparation of Reagents

Allow all reagents to reach room temperature.

Equalizing Reagent **EQUA-REAG**

Dissolve the lyophilized Equalizing Reagent by transferring the complete content of the Acylation Buffer ACYL-BUFF into the vial. Vortex briefly and leave on a roll mixer or orbital shaker for 20 minutes. Handle carefully in order to minimize foam formation. The reconstituted Equalizing Reagent should be stored frozen at -20 °C and is stable until the expiry date.

Wash Buffer **WASH**

Dilute the content (20 ml) of one bottle 50x concentrated Wash Buffer with distilled water to a total volume of 1000 ml, mix briefly. For further use the diluted wash buffer must be stored at 2 - 8 °C for a maximum period of 4 weeks.
Should the kit be used in several runs, then prepare only the required amount of wash buffer for each run.

Acylation Reagent **ACYL-REAG**

Remove the required amount of vials of Acylation Reagent from the foil pouch, leave the remaining vials inside together with the desiccant and close the pouch carefully.
Dissolve the content of one bottle in 3 ml SOLVENT and mix for at least 5 minutes on a roll mixer or orbital shaker. The Acylation Reagent has always to be prepared immediately before use (minimum stable for 3 hours). After use the reagent has to be discarded.
The two additional bottles are allowing a second and a third run of the test. If the whole kit is to be used in one run it is recommended to pool the dissolved contents of two vials of Acylation Reagent.

All other reagents are ready for use.

7. Test Procedure

7.1 Preparation of Urine Samples

Allow all reagents to reach room temperature.
Duplicates are recommended.

The wells of the Preparation Plate should be used only once. Please mark the respective wells before using.

1. Pipette each **20 µl Standard 1 - 6** CAL 1 – 6 **, Control 1 & 2** CON 1 & 2 **, and Urine Sample** into the respective wells of the Preparation Plate PRE-PLATE .

2. Pipette each **300 µl Equalizing Reagent** EQUA-REAG into all wells.

3. Incubate for 5 minutes at room temperature on an orbital shaker (medium shaking rate).

   Take each 20 µl for the ELISA.

7.2 Elisa

Allow all reagents to reach room temperature.

1. Pipette each **20 µl diluted Standards 1 to 6, Controls and Urine Samples** from the Preparation Plate into the respective wells of the coated Microtiter Strips [STRIPS].
   Leave remaining microtiter strips in the foil pouch together with the desiccant and close carefully.

2. Pipette each **50 µl freshly prepared Acylation Reagent** [ACYL-REAG] into all wells and continue with the next step, **immediately.**

3. Incubate for 20 minutes at room temperature on an orbital shaker (medium shaking rate)..

4. Pipette each **50 µl Antiserum** [AS] into all wells.
   Please use an Eppendorf Multipette or similar device (no single-channel or multi-channel pipettes).

5. Incubate for 30 minutes at room temperature on an orbital shaker (medium shaking rate).

6. Discard or aspirate the contents of the wells and wash thoroughly with each **prepared 300 µl Wash Buffer**. Repeat the washing procedure 3 times. Remove residual liquid by tapping the inverted plate on clean absorbent paper.

7. Pipette each **100 µl Enzyme Conjugate** [CONJ] into all wells.

8. Incubate for 20 minutes at room temperature on an orbital shaker (medium shaking rate).

9. Washing: Repeat step 6.

10. Pipette each **100 µl Substrate** [SUB] into all wells.

11. Shake on an orbital shaker for 10 seconds and then incubate for 20 ± 5 minutes at room temperature (20 - 25 °C), without shaking, on the table, cover plate with a box.

12. Pipette each **100 µl Stop Solution** [STOP] into all wells.
    Shake on an orbital shaker for 10 seconds

13. Read the optical density at 450 nm (reference wavelength between 570 and 650 nm) in a microplate photometer, within 15 minutes.

8. Calculation of the Results

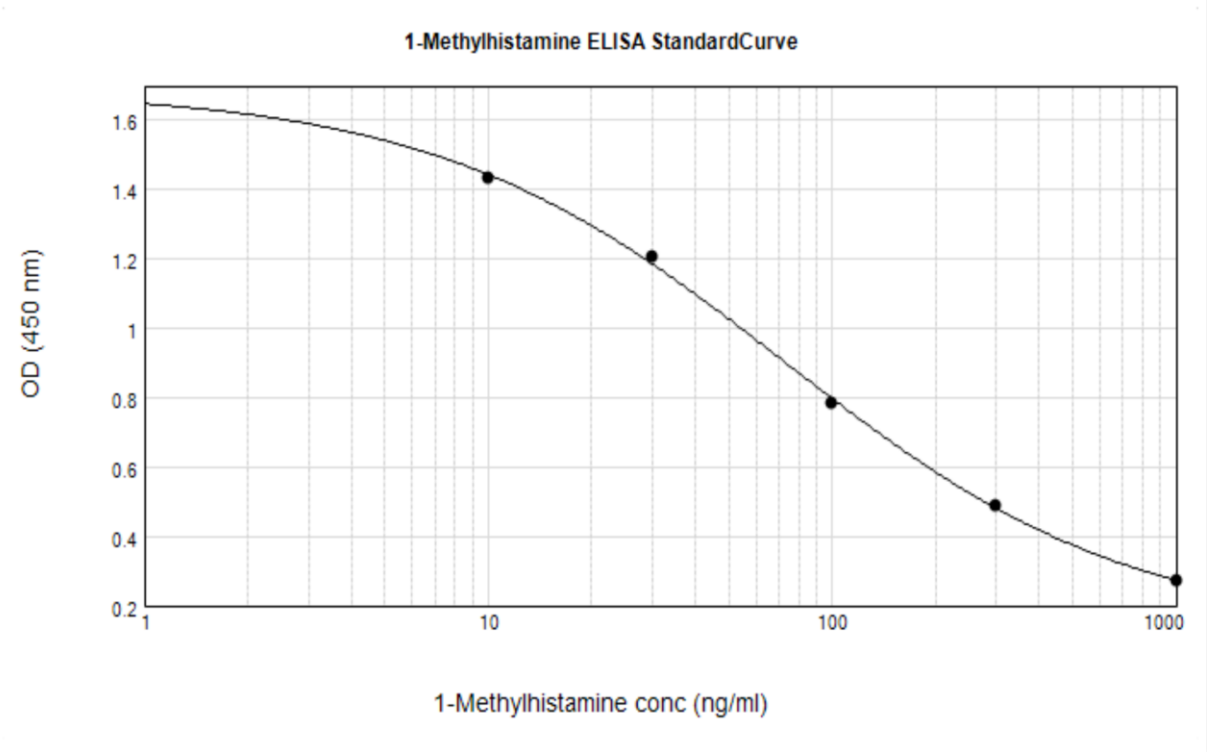
On a semilogarithmic graph paper the concentration of the standards (10 / 30 / 100 / 300 / 1000 ng/ml) (x-axis, logarithmic) are plotted against their corresponding optical density (y-axis, linear). Alternatively, the optical density of each standard and sample can be related to the optical density of the zero standard, expressed as the ratio OD/OD_{max}, and then plotted on the y-axis.

A good fit is provided with 4 Parameter Logistic (alternatively Log-Logit or Cubic Spline).

The concentration of the controls and urine samples can be read directly from this standard curve by using their average optical density.

Conversion:
1-Methylhistamine: 1 ng / ml = 8.0 nmol / l

Typical standard curve:



Quality control

All kit controls must be found within the acceptable ranges as printed on the q.c. certificate. If the criteria are not met, the run is not valid and should be repeated.

9. Assay Characteristics

9.1 Reference Range

The reference ranges given below should only be taken as a guideline. It is recommended that each laboratory should establish its own normal values.

Matrix	Reference Range
Urine, 24 h	< 250 µg/day
Urine, spontaneous	30 - 200 µg / g creatinine

9.2 Sensitivity

Matrix	Lower Detection Limit	Calculation
Urine	3.0	OD_{Cal1} - 2xSD

9.3 Specificity (Cross Reactivity)

Substance	Cross Reactivity (%)
1-Methylhistamine	100
Histamine	< 0.7
3-Methylhistamine	< 0.07
1-Methyl-4-imidazole acetic acid	< 0.0025
Imidazole-4-acetic acid	< 0.007
L-Histidine	< 0.0025

9.4 Recovery after Spiking

Matrix	Range (ng/ml)	Mean (%)	Range (%)
Urine	51 - 324	97	92 - 100

9.5 Linearity (recovery after dilution with dist. water)

Matrix	Range (ng/ml)	Highest Dil.	Mean (%)	Range (%)
Urine	33 - 339	1 : 10	102	97 - 107

9.6 Reproducibility

Matrix	Range (ng/ml)	Intra-Assay-CV
Urine	52 – 190	6.7 – 6.9 %

9.7 Comparison of Methods

Matrix	Method	Correlation
Urine	LC/MS	Y = 0.93 x LC/MS - 9.1; R = 0.993; N = 32

9.8 Calibration

The assay is calibrated by weighing in the pure substance. The correctness of the method was verified by comparison of method (see 9.7).

9.9 Limitations of Method

This assay is a diagnostic aid. A definite clinical diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical and laboratory findings have been evaluated concerning the entire.
Samples showing concentrations above the highest standard have to be diluted with dist. water (see 9.5) and reassayed. The values of diluted samples must be multiplied by the appropriate dilution factor.

9.10 Interfering Substances

Do not use collected urine samples which are not acidified.

10. Literature

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11. Symbols

I V D	In-Vitro-Diagnostic Medical Device	CE	CE marked
CONT	Content		Expiry Date
LOT	Lot number		Store at
	Manufactured by		Sufficient for …. determinations
REF	Catalogue number		Consult instructions for use

Pipetting Scheme

Preparation of Urine Samples

PRE-PLATE		Standard	Control	Urine Sample
Standard 1 - 6	µl	20		
Control 1 & 2	µl		20	
Urine Sample	µl			20
Equalizing Reagent	µl	300	300	300

Shake for 5 minutes at room temperature

Take each 20 µl for the ELISA

ELISA

STRIPS		Diluted Standard	Diluted Control	Diluted Urine Sample
Diluted Standard	µl	20		
Diluted Control	µl		20	
Diluted Urine Sample	µl			20
Acylation Reagent	µl	50	50	50

Immediately: Shake for 20 minutes at room temperature

Antiserum	µl	50	50	50

Shake for 30 minutes at room temperature

4 x washing

Enzyme Conjugate	µl	100	100	100

Shake for 20 minutes at room temperature

4 x washing

Substrate	µl	100	100	100

Shake for 10 seconds
Incubate for 20 ± 5 minutes at room temperature, covered (box), without shaking

Stop Solution	µl	100	100	100

Shake for 10 seconds
Reading of absorbance at 450 nm within 15 minutes

Symbols / Symbole / Symboles / Símbolos / Simboli / Símbolos / Σύμβολα

REF	Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.–Cat.: / N.º Cat.: / Αριθμός-Κατ.:
LOT	Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lotto n.: / Lote N.º: / Αριθμός -Παραγωγή:
	Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Da utilizzare entro:/ Usar até: / Χρησιμοποιείται από:
Σ	No. of Tests: / Kitgröße: / Nb. de Tests: / No. de Determ.: / Quantità dei tests: / N.º de Testes: / Αριθμός εξετάσεων:
CONC	Concentrate / Konzentrat / Concentré / Concentrar / Concentrato / Concentrado / Συμπύκνωμα
LYO	Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizzato / Liofilizado / Λυοφιλιασμένο
IVD	In Vitro Diagnostic Medical Device / In-vitro-Diagnostikum / Appareil Médical pour Diagnostics In Vitro / Dispositivo Médico para Diagnóstico In Vitro / Dispositivo Medico Diagnostico In vitro / Equipamento Médico de Diagnóstico In Vitro / Ιατρική συσκευή για In-Vitro Διάγνωση
BIO	Contains biological material of human origin / Enthält biologisches Material menschlichen Ursprungs / Contient une substance biologique d'origine humaine / Contiene material biológico de origen humano / Contiene materiale biologico di origine umana / Contém material biológico de origem humana / Περιέχει βιολογικό υλικό ανθρώπινης προέλευσης
BIO	Contains biological material of animal origin / Enthält biologisches Material tierischen Ursprungs / Contient une substance biologique d'origine animale / Contiene material biológico de origen animal / Contiene materiale biologico di origine animale / Contém material biológico de origem animal / Περιέχει βιολογικό υλικό ζωικής προέλευσης
UDI	Unique Device Identification / Eindeutige Gerätekennung / Identifiant de dispositif unique / Identificación única de producto / Identificatore univoco del dispositivo / Identificador de dispositivo único / Μοναδικός αναγνωριστικός κωδικός προϊόντος
	Read instructions before use / Arbeitsanleitung lesen / Lire la fiche technique avant emploi / Lea las instrucciones antes de usar / Leggere le istruzioni prima dell'uso / Ler as instruções antes de usar / Διαβάστε τις οδηγίες πριν την χρήση
	Keep away from heat or direct sun light / Vor Hitze und direkter Sonneneinstrahlung schützen / Garder à l'abri de la chaleur et de toute exposition lumineuse / Manténgase alejado del calor o la luz solar directa / Non esporre ai raggi solari / Manter longe do calor ou luz solar directa / Να φυλάσσεται μακριά από θερμότητα και άμεση επαφή με το φως του ηλίου
	Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazenar a: / Conservare a: / Armazenar em: / Αποθήκευση στους:
2-8°C	Store at: 2 - 8°C / Lagern bei: 2 - 8°C / Stocker à: 2 - 8°C / Almacene a: 2 - 8°C / Armazenar a: 2 - 8°C / Conservare a: 2-8°C / Armazenar em: 2-8°C / Αποθήκευση στους: 2-8°C
	Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbricante: / Παραγωγός:
	Distributor: / Distributor: / Distributeur: / Distributor: / Distributore: / Distribuidor: / Διανομέας:
	Caution! / Vorsicht! / Attention! / ¡Precaución! / Attenzione! / Cuidado! / Προσοχή!
	Symbols of the kit components see MATERIALS SUPPLIED. Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben. Voir MATERIEL FOURNI pour les symbôles des composants du kit. Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS. Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT. Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS. Για τα σύμβολα των συστατικών του κιτ συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.

Generic table, not all symbols are present in the product

COMPLAINTS: Complaints may be submitted initially written or vocal. Subsequently they need to be filed including the test performance and results in writing in case of analytical reasons.

WARRANTY: The product is warranted to be free from material defects within the specific shelf life and to comply with product specifications delivered with the product. The product must be used according to the Intended use, all instructions given in the instructions for use and within the product specific shelf life. Any modification of the test procedure or exchange or mixing of components of different lots could negatively affect the results. These cases invalidate any claim for replacement.

LIMITATION OF LIABILITY: IN ALL CIRCUMSTANCES THE EXTENT OF MANUFACTURER'S LIABILITY IS LIMITED TO THE PURCHASE PRICE OF THE KIT(S) IN QUESTION. IN NO EVENT SHALL MANUFACTURER BE LIABLE FOR ANY INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING DAMAGES FOR LOST PROFITS, LOST SALES, INJURY TO PERSON OR PROPERTY OR ANY OTHER INCIDENTAL OR CONSEQUENTIAL LOSS.

The labelling of hazardous substances is according to European directive.
For further country-specific classifications, please refer to the corresponding safety data sheet.

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