DIAGNOSTIKA GMBH

Instruction for use

1-Methylhistamine ELISA

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

RUO

For Research Use Only
Not for Use in Diagnostic Procedures

REF	EA208/96
Σ	12 x 8
	2 – 8 °C

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Symbols

RUO	For Research Use Only		
CONT	Content		Expiry Date
LOT	Lot Number		Store at
	Manufactured by		Sufficient for ... determinations
REF	Catalogue Number		Consult Instructions for Use

1 Introduction and Principle of the Test

Histamine (nomenclature: 2-(4-imidazolyl)-ethylamine) is a natural substance that is widespread in the human and animal organism. It is easily soluble in water and has a basic character. Biochemically, histamine is one of the biogenic amines and is formed from the amino acid histidine. This decarboxylation takes place with the help of the enzyme histidine decarboxylase. The biosynthesis of histamine takes place in the mast cells, cells of the epidermis and gastric mucosa and in the nerve cells.

Histamine can be released in a burst from mast cells and basophilic granulocytes when the storage cells are stimulated with the corresponding allergen. This stimulation occurs through the binding of the allergen to the specific IgE antibodies on the surface of the target cells. However, this effect does not occur upon first contact with an allergen. Initial contact leads to the formation of plasma cells that produce and release specific IgE antibodies. These bind to the corresponding receptors of the mast cells (sensitization). At subsequent exposure to the allergen, the allergen binds directly to the IgE antibodies of the mast cells, triggering the spontaneous release of histamine from the granules of the mast cells (immediate allergic reaction).

Circulating histamine is rapidly converted into 1-methylhistamine (N-methylhistamine) by histamine-N-methyltransferase. Excretion occurs into the urine and therefore the determination of this metabolite in the urine is of interest.

This assay is for research use only! Not for use in diagnostic procedures!

The 1-Methylhistamine ELISA Kit contains reagents for the quantitative determination of derivatized 1-methylhistamine in human urine samples. After sample preparation in the preparation plate, derivatization is carried out in the ELISA plate. In this process, 1-methylhistamine is quantitatively converted into N-acyl-1-methylhistamine by the acylation reagent.

The 1-methylhistamine ELISA is a competitive enzyme immunoassay. Antigen bound to the solid phase and free antigen in solution compete for a defined number of antibody binding sites. When the system is in equilibrium, the unbound antigen-antibody complex is removed in a washing step and the correspondingly bound complex is detected using a peroxidase conjugate and determined via the conversion of tetramethylbenzidine (TMB). The TMB/POD reaction is stopped and measured at 450 nm. The concentration of the

antigen-antibody complex bound to the solid phase is inversely proportional to the concentration of the antigen in the sample.

2 Precautions

- For research use only. Not for use in diagnostic procedures.
- Before carrying out the test, the valid instructions 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 have been obtained from certified healthy animals but these materials should be handled as potentially infectious.
- Individual components of different lots and test kits should not be interchanged. The expiry dates and storage conditions stated on the packaging and the labels of the individual components must be observed.
- When handling the reagents, controls and samples, the current laboratory safety guidelines and good laboratory practice should be observed.
- Wear protective clothing, disposable gloves, and safety goggles while performing the test.
- According to the CLP Regulation No. 1272/2008, the kit components do not have to be labelled as hazardous substances. Detailed safety information can be found in the safety data sheet.
- Avoid any actions that could result in ingestion, inhalation or injection of the reagents. Never pipette by mouth.
- Avoid contact with individual reagents.
- Dispose of waste according to state and local environmental protection regulations.
- 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.

Broken glass can cause injury. Be cautious with glass vials.

3 Storage and Stability

The kit is shipped at ambient temperature. Upon 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 shelf life of the ready-to-use reagents is indicated on the respective bottle label. For stability of prepared reagents refer to 6.

Reagents must equilibrate to room temperature before use and refrigerated immediately after use.

4 Contents of the Kit

MT-Strips **STRIPS** 12 strips

8 wells each, break apart
precoated with N-acyl-1-methylhistamine

Standards 1 - 6 **CAL 1 – 6** 6 vials

Each 4 ml, ready for use
Concentrations:

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

Control 1 & 2 **CON 1 & 2** 2 vials

4 ml each, ready for use
Range: see QC certificate

Acylation Buffer **ACYL-BUFF** 1 vial

32 ml, ready for use, color coded blue

Acylation Reagent **ACYL-REAG** 3 vials

3 ml lyophilized, dissolve with SOLVENT

Antiserum **AS** 1 vial

6 ml, ready for use, color coded yellow
Rabbit-anti-N-acyl-1-methylhistamine

Enzyme Conjugate 13 ml, ready for use Goat anti-rabbit-IgG-peroxidase	**CONJ**	1 vial
Wash Buffer 20 ml, concentrated (50x)	**WASH**	1 vial
Substrate 13 ml TMB solution, ready for use	**SUB**	1 vial
Stop Solution 13 ml, ready for use contains 0.3 M sulphuric acid	**STOP**	1 vial
Preparation Plate for sample preparation	**PRE-PLATE**	2 pieces
Equalizing Reagent lyophilized, dissolve with 32 ml ACYL-BUFF	**EQUA-REAG**	1 vial
Solvent 11 ml, ready for use, color coded yellow	**SOLVENT**	1 vial

Additional materials and equipment required but not provided:

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

5 Sample Collection

Avoid repeated freezing and thawing of the samples.

Spontaneous urine can be used for this test as well as collected urine.

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 warnings) as preservative. Determine the total volume and take an aliquot for the measurement. For donors with suspected kidney disorders the creatinine concentration should be determined, too. Urine samples can be stored at -20 °C for at least 6 months.

Mix and centrifuge urine before use.

6 Preparation of Reagents

Equilibrate reagents to room temperature

6.1 Equalizing Reagent

Reconstitute the lyophilized Equalizing Reagent EQUA-REAG by transferring the complete content of the Acylation Buffer ACYL-BUFF into the vial. Vortex briefly and mix for at least 20 minutes on a roller mixer or similar shaker until completely dissolved. Thereby, avoid excessive formation of foam. The reconstituted Equalizing Reagent should be stored frozen at -20 °C and is stable until the expiry date.

6.2 Wash Buffer

Dilute the content (20 ml) of 50x concentrated Wash Buffer WASH with dist. water to a total volume of 1,000 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.

6.3 Acylation Reagent

Remove the required amount of vials of Acylation Reagent ACYL-REAG from the foil pouch, leave the remaining vials inside together with the desiccant and close the pouch carefully. Reconstitute each vial of lyophilized Acylation Reagent with 3 mL of Solvent SOLVENT and mix on a roller mixer or similar shaker for at least 5 minutes. The Acylation Reagent should be freshly prepared immediately before the performing the test and is then stable for approx. 3 hours. The kit contains 4 vials of Acylation Reagent for multiple runs. When using the kit in one run, pool the dissolved contents of two vials. Discard the remaining reconstituted reagent after use.

All other reagents are ready for use.

7 Test Procedure

7.1 Preparation of Urine Samples

Duplicates are recommended. The wells of the Preparation Plate PRE-PLATE should be used only once. Please mark the respective wells before use.

1. **Pipette**
   20 µl Standards 1 – 6 CAL 1 – 6,
   20 µl Controls 1 & 2 CON 1 & 2 and
   20 µl Urine,
   into the respective wells of the Preparation Plate PRE-PLATE.
2. **300 µl Equalizing Reagent** EQUA-REAG (s. 6.1) into each well.
3. Incubate for 5 minutes at room temperature on a orbital shaker at medium frequency.

Take **20 µl** each for the ELISA.

7.2 ELISA Urine Samples

1. Pipette **20 µl each of diluted standards, controls and samples** from the Preparations Plate [PRE-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 **50 µl Acylation Reagent** [ACYL-REAG] into each well and continue with step 3, immediately.
3. Incubate for 20 minutes at room temperature on an orbital shaker at medium frequency.
4. Pipette **50 µl Antiserum** [AS] into each well. Please use Multipette or similar (no single-channel or multi-channel pipettes).
5. Incubate for 30 minutes at room temperature (20 – 25 °C) on an orbital shaker at medium frequency.
6. Discard or aspirate the contents of the wells and wash thoroughly with **300 µl diluted Wash Buffer** [WASH] per well. Discard or aspirate the contents of the wells and remove residual liquid by tapping the inverted plate on a clean absorbent paper. Repeat the washing procedure 4 times. Alternatively, a washing device may be used.
7. Pipette **100 µl Enzyme Conjugate** [CONJ] into each well.
8. Incubate for 20 minutes at room temperature on an orbital shaker at medium frequency.
9. Wash: Repeat step 6.
10. Pipette **100 µl Substrate** [SUB] into each well.
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 large box.
12. Pipette **100 µl Stop Solution** [STOP] into each well.
    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).

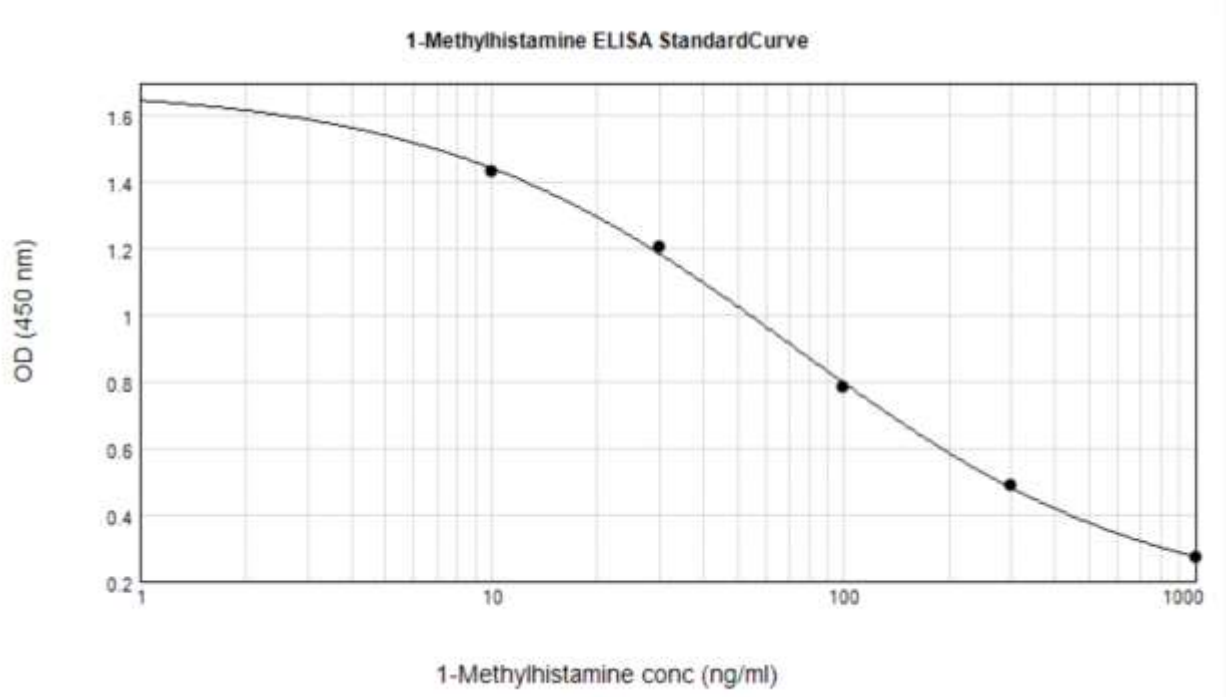
A good fit is provided with 4 Parameter Logistic (alternatively Log-Logit or Cubic Spline).

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

Conversion factor:

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

Typical standard curve:



Quality Control: Test results are valid only if the kit controls are within the ranges specified on the QC Certificate. Otherwise, the test should be repeated.

9 Assay Characteristics

9.1 Reference Range

This kit is for research use only, the values below are not for use in diagnostic procedures and should only be taken as a guideline. It is recommended that each laboratory establishes its own normal values.

Matrix	Reference Range
Urine, 24h	< 250 µg/day
Urine, spontanous	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 calibration is carried out by weighing the pure substance. The correctness of the method was determined by comparison of method (9.7).

9.9 Limitations of Method

Samples measured above the highest standard must be diluted with distilled water and reassayed. The values of diluted samples must be multiplied by the appropriate dilution factor.

9.10 Interferences

Do not use non-acidified urine collection.

10 Changes to declare

Version _7: Extensive changes have been made and are highlighted in grey. Cell culture samples and plasma as a matrix have been removed.

Version _6: IFU has been re-formatted. Component names as printed on labels were included in sections 6, 7 and 8 and pipetting schemes to provide greater clarity. No changes have been made to components or execution of protocols.

11 Literature

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Pipetting Scheme

Preparation of Urine Samples

		Standard	Control	Urine Sample
PRE-PLATE:				
CAL 1 – 6	µl	20		
CON 1 & 2	µl		20	
Urine Sample	µl			20
EQUA-REAG	µl	300	300	300

Shake plate for 5 minutes
Take 20 µl each for the ELISA

ELISA

		Diluted Standards	Diluted Controls	Diluted Samples
STRIPS:				
Transfer from PRE-PLATE into STRIPS	µl	20	20	20
ACYL-REAG	µl	50	50	50

Immediately, shake for 20 minutes at room temperature

AS	µl	50	50	50

Shake for 30 minutes at room temperature
4 x washing

CONJ	µl	100	100	100

Shake for 20 minutes at room temperature
4 x washing

SUB	µl	100	100	100

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

STOP	µl	100	100	100

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