DIAGNOSTIKA GMBH

Instructions for Use

1-Methylhistamine Plasma / Cell Culture - Kit

Complementary Kit enabling the
Quantitative Determination of
1-Methylhistamine (N-Methylhistamine)
in Plasma and Cell Culture

RUO

For Research Use Only
Not for Use in Diagnostic Procedures

REF	EA215/96
Σ	96
+2 +8 °C	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

Hazard Pictograms

Warning

Danger

1 Introduction and Principle of the Test

The 1-Methylhistamine Plasma / Cell Culture - Kit (EA215/96) is a complementary kit to the 1-Methylhistamine ELISA (EA208/96) enabling quantitative determination of 1-Methylhistamine in EDTA and Heparin plasma and cell culture samples.

In a first step, using this kit, proteins and protein-like structures are removed from the samples by precipitation.

In the following, using the 1-Methylhistamine ELISA kit (EA208/96), the 1-Methylhistamine is quantitatively acylated to N-acyl-1-Methylhistamine and the ELISA performed.

The 1-Methylhistamine ELISA is a competitive enzyme immunoassay in which antigens compete for a defined number of antibody binding sites. When the system is in equilibrium, unbound antigen-antibody complexes are removed in a wash step and the corresponding bound complexes are detected using a peroxidase conjugate and determined by the turnover of tetramethylbenzidine (TMB). The TMB/POD reaction is stopped and the OD 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.

NOTE: When determining 1-Methylhistamine in EDTA and Heparin plasma and cell culture samples refer to this manual only. Do not use the manual included in the 1-Methylhistamine ELISA kit (EA208/96).

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.
- 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.
- Some components of this kit contain hazardous reagents. These components are marked with the adequate hazard label. Further information is in section 4 and in the corresponding MSDS.
- 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.

3 Storage and Stability

The kit is shipped at ambient temperature and is subsequently stable until its expiry date when stored at 2 - 8 °C. Once opened, the kit is stable until its expiry date.

The shelf life of the ready-to-use reagents is indicated on the respective vial label. For shelf life and storage conditions of prepared reagents refer to 6.1.

Bring reagents to room temperature before use and refrigerate immediately after use.

4 Contents of the Kit

Precipitation Tube — **PRECI-TUBE** — 100 pieces
Ready for use

Precipitator 1 — **PRECI 1** — 1 vial
3 ml, ready for use


Warning

Precipitator 2 — **PRECI 2** — 1 vial
3 ml, ready for use, corrosive


Danger

Adhesive Foil — **FOIL** — 6 pieces
Ready for use

Additional materials and equipment required but not included:

- 1-Methylhistamine ELISA (EA208/96)
- Pipettes (20, 30, 40, 50, 80, and 100 µl)
- Multichannel pipette or Microplate washing device
- Multipette
- Distilled water
- Microplate photometer (450 nm)
- Horizontal shaker
- Vortex mixer and roller mixer
- Paper towels, pipette tips, timer
- Centrifuge
- Polypropylene reaction tubes for diluting standards and controls

5 Sample Collection

Avoid repeated freezing and thawing of the samples.

5.1 Plasma

EDTA plasma and heparin plasma can be used for the test.

Samples can be stored at 2 – 8 °C for up to 6 hours. Samples that are not immediately used in the assay can be stored at -20 °C for at least 6 months.

5.2 Cell culture

Cell culture media DMEM (+ 10 % Fetal Calf Serum) and RPMI 1640 (+ 10 % Fetal Calf Serum) were used for validation.

Other cell culture media are to be tested by the user.

6 Preparation of Reagents and Samples

6.1 Preparation of Reagents

Bring kit contents to room temperature. The EQUA-REAG is not required.

6.1.1 Wash Buffer

Dilute the content (20 ml) of the 50x concentrated Wash Buffer WASH with dist. water to a total volume of 1,000 ml, mix briefly. The diluted wash buffer can be stored at 2 – 8 °C for a maximum period of 4 weeks.

Should the kit be used in several runs, it is recommended to prepare only the required amount of wash buffer for each run.

6.1.2 Acylation Reagent

Remove the required amount of vials of Acylation Reagent ACYL-REAG from the foil pouch. Leave the remaining vials together with the desiccant inside the pouch 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 performing the test and is then stable for approx. 3 hours. The kit contains 3 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.

6.2 Preparation of Samples

With a permanent marker label the precipitation tubes PRECI-TUBE (one tube per standard, control and sample) and wells of the preparation plate PRE-PLATE (two wells per standard, control and sample, as duplicate determinations are recommended) and do not re-use.

When determining Plasma samples:

Short before use, dilute the standards CAL 1 - CAL 6 and controls CON 1 & CON 2 1:100 with dist. water. Use polypropylene (PP) reaction tubes (not supplied). Do not dilute the samples.

When determining Cell Culture samples:

Short before use, dilute the standards CAL 1 - CAL 6 and controls CON 1 & CON 2 1:100 with the same cell culture medium as used for the samples, if possible. Use polypropylene (PP) reaction tubes (not supplied). Do not dilute the samples.

If this is not possible, dilute the standards and controls 1:100 in distilled water. In this case, the cell culture samples must be diluted 1:5 in distilled water before use. Multiply the result of the samples by the factor 5.

Pipetting scheme for 1:100 dilution of standards and controls:

	Final Concentration	**Volume Dilution Medium**	**Volume Original Standard**
CAL 1	**0 ng/ml**	990 µl	10 µl CAL 1 (0 ng/ml)
CAL 2	**0.1 ng/ml**	990 µl	10 µl CAL 2 (10 ng/ml)
CAL 3	**0.3 ng/ml**	990 µl	10 µl CAL 3 (30 ng/ml)
CAL 4	**1 ng/ml**	990 µl	10 µl CAL 4 (100 ng/ml)
CAL 5	**3 ng/ml**	990 µl	10 µl CAL 5 (300 ng/ml)
CAL 6	**10 ng/ml**	990 µl	10 µl CAL 6 (1000 ng/ml)
CON 1	**/**	990 µl	10 µl CON 1
CON 2	**/**	990 µl	10 µl CON 2

It is recommended to perform duplicate determinations. However, if single determinations are preferred, cut the volumes below in half, i.e. 40 µl each of diluted Standards and Controls and undiluted samples and 10 µl each of Precipitator 1 and 2. After centrifugation, take 1 x 30µl supernatant for acylation.

Precipitation procedure (one tube per Standard, Control and Sample):

1. Pipette **80 µl each of the diluted Standards and Controls and undiluted samples** into the labelled Precipitation Tubes PRECI-TUBE.
2. Add **20 µl Precipitator 1** PRECI 1 into each tube.
3. Add **20 µl Precipitator 2** PRECI 2 into each tube.
4. Mix the tubes vigorously (vortex).
5. Centrifuge tubes at 4,000 x g for 15 minutes.

Acylation (duplicate wells per Standard, Control and Sample):

6. Transfer **2 x 30 µl of each supernatant** into the corresponding wells (duplicates) of the labelled Preparation Plates PRE-PLATE
7. Add **50 µl Acylation Buffer** ACYL-BUFF into each well.
8. Shake for 10 seconds on a horizontal shaker.
9. Add **40 µl dissolved Acylation Reagent** ACYL-REAG into each well.
   Carefully seal plate with adhesive foil FOIL and proceed **immediately** to step 10.
10. Mix for 30 minutes at room temperature (20 – 25 °C) on a horizontal shaker at medium speed.
11. Add **20 µl Antiserum** AS into each well.
12. Carefully seal the plate with adhesive foil FOIL.
13. Mix for 120 minutes at room temperature (20 – 25 °C) on a horizontal shaker at medium speed.

7 Test Procedure ELISA

1. Transfer **100 µl each of prepared Standards, Controls and Samples** from the Preparation Plate PRE-PLATE into the corresponding wells of the Microtiter Strips STRIPS, thereby slightly tilting the Preparation Plate to facilitate pipetting.
   Leave unused Microtiter Strips in the foil pouch together with the desiccant and seal carefully.
2. Seal the wells of the Microtiter Strips with adhesive foil FOIL and shake for 10 seconds on a horizontal shaker.
3. Incubate for 15 - 20 hours (overnight) at 2 – 8 °C.
4. Discard or aspirate the contents of the wells and wash with approx. **300 µl diluted Wash Buffer** WASH per well. Empty wells and remove residual liquid by firmly tapping the inverted microtiter strips on a clean paper towel. Repeat the washing step 3 times.
   Alternatively, a washing device can be used.
5. Add **100 µl Enzyme Conjugate** CONJ into each well.
6. Mix for 30 minutes at room temperature on a horizontal shaker at medium speed.
7. Wash: Repeat wash step 4.
8. Pipette **100 µl Substrate** SUB into each well.
9. Shake for 10 seconds on a horizontal shaker.
10. Incubate for 25 ± 5 minutes at room temperature (20 – 25 °C) on the table covered with a box without shaking.
11. Pipette **100 µl Stop Solution** STOP into each well.
12. Shake for 10 seconds on a horizontal shaker.
13. Read absorbance in microtiter plate photometer at 450 nm (reference wavelength between 570 nm and 650 nm) within 15 minutes.

8 Calculation of the Results

The optical density (OD) of the standards (y-axis, linear) are plotted against the corresponding concentrations of the standards (x-axis, logarithmic).

When using software, a 4-Parameter Logistic is recommended (alternative: Cubic-Spline or Logit-Log).

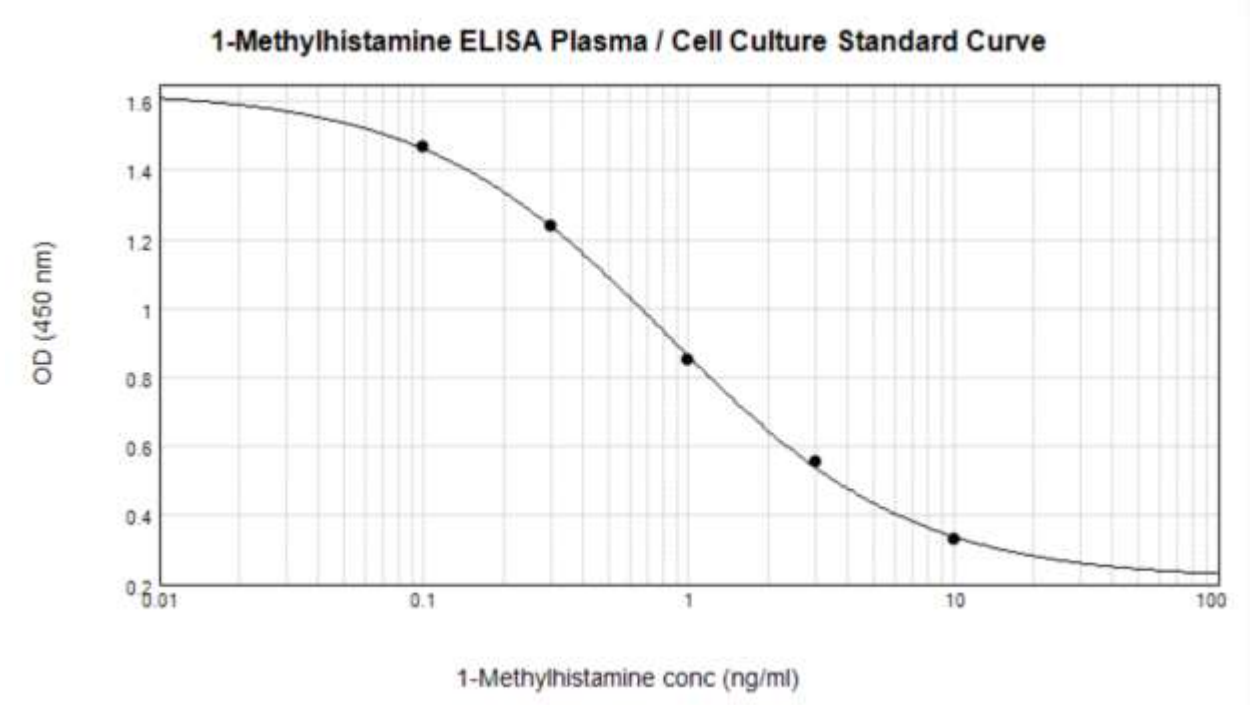
The concentrations of the controls in ng/ml must be multiplied by a factor of 100.

The concentrations of the samples can be read directly from the standard curve in ng/ml.

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

Standard	1	2	3	4	5	6
ng/ml	0	0.1	0.3	1	3	10
nmol/l	0	0.8	2.4	8	24	80

Typical example of standard curve (do not use for calculation of results):



9 Assay Characteristics

9.1 Sensitivity

Matrix	Lower Detection Limit	Calculation
Plasma, Cell Culture	0.029 ng/ml	OD_{CAL1} – 2xSD

9.2 Recovery after Spiking

Matrix	Range (ng/ml)	Mean (%)	Range (%)
EDTA-Plasma	0.32 – 2.81	93	91 - 94
Heparin-Plasma	0.26 – 4.36	103	94 - 107
Cell Culture (DMEM + 10% FCS)	0.32 – 2.79	94	90 - 101
Cell Culture (RPMI + 10% FCS)	0.31 – 2.60	92	91 - 95

9.3 Linearity (recovery after dilution with dist. water)

Matrix	Range (ng/ml)	Max. Dil.	Mean (%)	Range (%)
EDTA-Plasma	0.70 – 9.79	1 : 15	94	87 - 106
Heparin-Plasma	0.38 – 5.55	1 : 15	102	90 - 111

9.4 Linearity (recovery after dilution with cell culture medium)

Matrix	Range (ng/ml)	Max. Dil.	Mean (%)	Range (%)
Cell Culture (DMEM + 10% FCS)	0.81 – 3.74	1 : 5	104	95 - 108
Cell Culture (RPMI + 10% FCS)	0.82 – 4.19	1 : 5	94	90 - 98

9.5 Reproducibility

Intra-CV

Matrix	Range (ng/ml)	Intra-Assay-CV (%)
EDTA-Plasma	0.33 – 0.92	6.7 – 8.2
Zellkultur	0.37 – 1.29	9.9 – 8.1

Inter-CV

Matrix	Range (ng/ml)	Inter-Assay-CV (%)
EDTA-Plasma	0.31 – 0.81	9,9 – 8,9

10 Changes to declare

Version _2: Correction of section 1 as highlighted in gray.

Version _1: IFU issued

Pipetting Scheme – Sample Preparation

		Standard	Control	Sample
PRECI-TUBE (single):				
CAL 1 – 6 (dil. 1:100)	µl	80		
CON 1 & 2 (dil. 1:100)	µl		80	
Sample	µl			80
PRECI 1	µl	20	20	20
PRECI 2	µl	20	20	20

Vortex vigorously
Centrifuge 15 minutes at 4,000 x g

PRE-PLATE (duplicates):				
Transfer from PRECI-TUBE into PRE-PLATE	µl	30	30	30
ACYL-BUFF	µl	50	50	50

Shake for 10 seconds

ACYL-REAG	µl	40	40	40

Seal carefully with FOIL
Immediately, shake 30 minutes at RT

AS	µl	20	20	20

Seal carefully with FOIL
Shake 120 minutes at RT

Pipetting Scheme - ELISA

		Prepared Standards	Prepared Controls	Prepared Samples
STRIPS:				
Transfer from PRE-PLATE into STRIPS	µl	100	100	100

Seal carefully with FOIL
Shake for 10 seconds
Incubate 15 – 20 hours (overnight) at 2 – 8 °C

Wash 4x with 300 µl WASH per well

CONJ	µl	100	100	100

Shake 30 minutes at RT
Wash 4x with 300 µl WASH per well

SUB	µl	100	100	100

Shake for 10 seconds
Incubate 25 ± 5 minutes at RT, covered with a box, without shaking

STOP	µl	100	100	100

Shake for 10 seconds
Read absorbance at 450 nm (ref. 570 – 650 nm)