



EAGLE
BIOSCIENCES

1-Methylhistamine ELISA Assay Kit

Catalog Number:
MHU34-K01

For Research Use Only. Not for use in diagnostic procedures.
v. 1.0

EAGLE BIOSCIENCES, INC.
20A Northwest Blvd., Suite 112, Nashua, NH 03063
Phone: 617-419-2019 Fax: 617-419-1110
WWW.EAGLEBIO.COM



INTENDED USE

The Eagle Biosciences 1-Methylhistamine ELISA Assay kit is an enzyme-linked immunosorbent assay used for the quantitative and very sensitive determination of 1-Methylhistamine in biological samples including urine, plasma, and cell culture samples. The 1-Methylhistamine ELISA Assay kit is for research use only and should not be used in diagnostic procedures.

INTRODUCTION

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 is 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 role 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.

PRINCIPLE OF THE ASSAY

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.

PRECAUTIONS

- For research use only
- Disposable gloves and safety glasses should be used.
- 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.
- Some components of this kit are containing hazardous reagents. These components are marked with the adequate hazard label.

STORAGE AND STABILITY

On arrival, store the kit at 2-8 °C. Once opened the kit is stable until its expiry date. For stability of prepared reagents refer to Preparation of Reagents.

Do not use components beyond the expiration date shown on the labels.

Do not mix various lots of any kit component within an individual assay.



CONTENTS OF THE KIT

4.1 MT Strips 12 strips
8 wells each, break apart
Precoated with 1-methylhistamine

4.2 Standards 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
1-Methylhistamine (nmol/l)	0	80	240	800	2400	8000

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

4.4 Acylation Reagent 3 vials
Lyophilized, dissolve contents in 1.5 ml solvent
If necessary combine the contents of several vials

4.5 Solvent 1 vial
5.5 ml, ready for use
Contains Aceton and DMSO
Solvent to dissolve the acylation reagent

4.6 Antiserum 1 vial
5.5 ml, ready for use, colour coded blue
Rabbit-anti-N-acyl-1-methylhistamine

4.7 Enzyme Conjugate 1 vial
12 ml, ready for use
Goat anti-rabbit-IgG peroxidase

4.8 Wash Buffer 1 vial
20 ml, concentrate
Dilute content with dist. water to 500 ml total volume

4.9 Substrate 1 vial
12 ml TMB solution, ready for use

4.10 Stop Solution 1 vial
12 ml, ready for use
Contains 0.3 M sulphuric acid

4.11 Reaction Plate 1 piece
For acylation



- | | | |
|------|---|---------|
| 4.12 | Equalizing Reagent
Lyophilized, dissolve content with 20.5 ml diluent
Dissolve carefully to minimize foam formation | 1 vial |
| 4.13 | Diluent
20.5 ml, ready for use
Diluent to dissolve the equalizing reagent | 1 vial |
| 4.14 | Start Buffer
6 ml, ready for use | 1 vial |
| 4.15 | Adhesive Foil
Ready for use | 1 piece |

ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

- Pipettes 20, 50, 100, 200 μ l and Multipette
- Orbital shaker
- Multichannel pipette or Microplate washing device
- Microplate photometer (450 nm)
- Distilled water

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 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.

Plasma

The test can be performed with EDTA plasma. Hemolytic and lipemic samples should not be used. The samples can be stored for a few hours at 2 - 8 °C. For a longer storage (up to 6 months) the samples must be frozen at -20 °C. Repeated freezing and thawing should be avoided.

PREPARATION OF REAGENTS

Equalizing Reagent

Dissolve the contents with 20.5 ml DILUENT . Transfer the contents of the DILUENT vial completely, mix shortly and leave on a roll mixer or orbital shaker for 30 minutes. Handle carefully in order to minimize foam formation. The reconstituted Equalizing Reagent should be stored frozen at -20 °C and is stable for a minimum of 1 year.



Acylation Reagent

Dissolve the content of one bottle in 1.5 ml SOLVENT and shake for 5 minutes on a roll mixer or orbital shaker. The Acylation Reagent has always to be prepared immediately before use. 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 at least two vials of Acylation Reagent.

Please note that solvent reacts with many plastic materials including plastic trays; solvent does not react with normal pipette tips and with glass devices.

Solvent is volatile and the dissolved Acylation Reagent evaporates quickly. Therefore, please do not use a tray with big surface together with a multichannel pipette for pipetting Acylation Reagent. Rather, use an Eppendorf multipipette (or similar device), fill the syringe directly from the vial with dissolved Acylation Reagent and add well by well.

Wash Buffer

Dilute the content of one bottle with distilled water to a total volume of 500 ml. For further use the diluted wash buffer must be stored at 2 - 8 °C for a maximum period of 4 weeks.

All other reagents are ready for use.

TEST PROCEDURE URINE SAMPLES

Preparation of Urine Samples (Acylation)

Allow all reagents to reach room temperature.

Duplicates are recommended.

The wells of the reaction plate for the acylation can be used only once. Therefore, it is recommended to mark the respective wells before use.

1. Pipette each 20 µl Standard 1 - 6, Control 1 & 2 and Urine Sample into the respective wells of the reaction plate.
2. Pipette each 200 µl Equalizing Reagent (6.1.1) into all wells.
3. Pipette each 20 µl freshly prepared Acylation Reagent (6.1.2) into all wells and continue with step 4. immediately.

Colour changes to red!

Please note that solvent reacts with many plastic materials including plastic trays; solvent does not react with normal pipette tips and with glass devices.

Solvent is volatile and the dissolved Acylation Reagent evaporates quickly. Therefore, please do not use a tray with big surface together with a multichannel pipette for pipetting Acylation Reagent. Rather, use an Eppendorf multipipette (or similar device), fill the syringe directly from the vial with dissolved Acylation Reagent and add well by well.

4. Incubate for 60 minutes at room temperature on an orbital shaker (medium shaking rate). Do not cover the wells or the plate; leave the plate open on the shaker.

Take each 20 µl for the ELISA.



ELISA Urine Samples

Allow all reagents to reach room temperature.

1. Pipette each 50 μ l Start Buffer into the respective wells of the coated microtiter strips.
2. Pipette each 20 μ l prepared Standards 1 to 6, Controls and Urine Samples into the respective wells of the coated microtiter strips. Colour changes to red.
3. Pipette each 50 μ l Antiserum into all wells.
4. Incubate for 30 minutes at room temperature on an orbital shaker (medium shaking rate). Do not cover the wells or the plate; leave the plate open on the shaker.
5. Discard or aspirate the contents of the wells and wash thoroughly with each prepared 250 μ l Wash Buffer. Repeat the washing procedure 3 times. Remove residual liquid by tapping the inverted plate on clean absorbent paper.
6. Pipette each 100 μ l Enzyme Conjugate into all wells.
7. Incubate for 20 minutes at room temperature on an orbital shaker (medium shaking rate).
8. Washing: Repeat step 5.
9. Pipette each 100 μ l Substrate into all wells.
10. Incubate for 15 to 20 minutes at room temperature (20 - 25 $^{\circ}$ C) on an orbital shaker (medium shaking rate). Avoid exposure to direct sun light.
11. Pipette each 100 μ l Stop Solution into all wells.
12. Read the optical density at 450 nm (reference wavelength between 570 and 650 nm) in a microplate photometer within 15 minutes.

TEST PROCEDURE PLASMA AND CELL CULTURE SAMPLES

Preparation of Plasma and Cell Culture Samples (Acylation)

Allow all reagents to reach room temperature.

Duplicates are recommended.

The wells of the reaction plate for the acylation can be used only once. Therefore, it is recommended to mark the respective wells before use.

Prepare the following 1:50 dilutions of Standards and Controls in suitable polypropylene tubes or Eppendorf cups just prior to assay.

After use the dilutions have to be discarded.

	Conc.	Vol. dist. water	Vol. kit standard
Std 1	0 ng/ml	1000 μ l	/
Std 2	0.2 ng/ml	980 μ l	20 μ l CAL 2 (10 ng/ml)
Std 3	0.6 ng/ml	980 μ l	20 μ l CAL 3 (30 ng/ml)
Std 4	2 ng/ml	980 μ l	20 μ l CAL 4 (100 ng/ml)
Std 5	6 ng/ml	980 μ l	20 μ l CAL 5 (300 ng/ml)
Std 6	20 ng/ml	980 μ l	20 μ l CAL 6 (1000 ng/ml)
Control 1	/	980 μ l	20 μ l CON 1
Control 2	/	980 μ l	20 μ l CON 2

Samples are not to be diluted



1. Pipette each 50 μ l diluted Standard 1 - 6, diluted Control 1 & 2 and undiluted plasma and cell culture samples into the respective wells of the reaction plate.
2. Pipette each 100 μ l Equalizing Reagent (6.1.1) into all wells.
3. Pipette each 10 μ l freshly prepared Acylation Reagent (6.1.2) into all wells and continue with step 4. immediately. Colour changes to red! Please note that solvent reacts with many plastic materials including plastic trays; solvent does not react with normal pipette tips and with glass devices. Solvent is volatile and the dissolved Acylation Reagent evaporates quickly. Therefore, please do not use a tray with big surface together with a multichannel pipette for pipetting Acylation Reagent. Rather, use an Eppendorf multipipette (or similar device), fill the syringe directly from the vial with dissolved Acylation Reagent and add well by well.
4. Incubate for 60 minutes at room temperature on an orbital shaker (medium shaking rate). Do not cover the wells or the plate; leave the plate open on the shaker.

Take each 50 μ l for the ELISA.

ELISA Plasma and Cell Culture Samples

Allow all reagents to reach room temperature.

1. Pipette each 50 μ l Start Buffer into the respective wells of the coated microtiter strips.
2. Pipette each 50 μ l prepared Standards 1 to 6, Controls and plasma and cell culture samples into the respective wells of the coated microtiter strips. Colour changes to red.
3. Pipette each 20 μ l Antiserum into all wells.
4. Cover the plate with adhesive foil, shake plate briefly and incubate for 15 – 20 hours (overnight) at 2 – 6 °C.
5. Discard or aspirate the contents of the wells and wash thoroughly with each prepared 250 μ l Wash Buffer. Repeat the washing procedure 3 times. Remove residual liquid by tapping the inverted plate on clean absorbent paper.
6. Pipette each 100 μ l Enzyme Conjugate into all wells.
7. Incubate for 20 minutes at room temperature on an orbital shaker (medium shaking rate).
8. Washing: Repeat step 5.
9. Pipette each 100 μ l Substrate into all wells.
10. Incubate for 15 to 20 minutes at room temperature (20 - 25 °C) on an orbital shaker (medium shaking rate). Avoid exposure to direct sun light.
11. Pipette each 100 μ l Stop Solution into all wells.
12. Read the optical density at 450 nm (reference wavelength between 570 and 650 nm) in a microplate photometer within 15 minutes.

CALCULATION OF THE RESULTS

Calculation of Urine Samples

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).

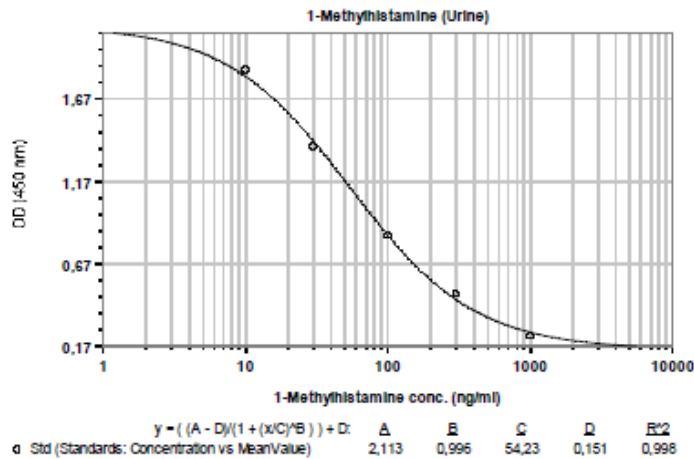
The concentration of the controls and urine samples can be read directly from this standard curve by using their average optical density.



Conversion factor:

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

Typical Standard Curve:



Calculation of Plasma and Cell Culture Samples

On a semilogarithmic graph paper the concentration of the diluted standards (0.2 / 0.6 / 2 / 6 / 20 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/ODmax and then plotted on the y-axis.

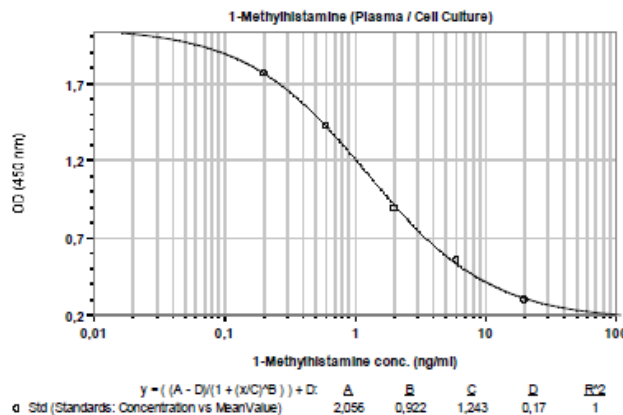
A good fit is provided with 4 Parameter Logistic (alternatively Log-Logit or Cubic Spline).

The concentration of the plasma and cell culture samples can be read directly from this standard curve by using their average optical density. The read concentrations of the controls have to be multiplied by 50.

Conversion factor:

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

Typical Standard Curve





ASSAY CHARACTERISTICS

Normal Range

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

Urine
<130 ng/ml

Sensitivity

The lower limit of detection was determined by taking the 3fold standard deviation of the absorbance of the Zero Reference and reading the corresponding value from the standard curve.

Urine	Plasma/Cell Culture
2.5 ng/ml	0.13 ng/ml

Specificity (Cross Reactivity)

Structural related components were tested for possible interference with the antisera against 1-methylhistamine used in the ELISA method.

Components	Cross Reactivity (%)
1-Methylhistamine	100
Histamine	0.39
3-Methylhistamine	0.014
Imidazole-4-acetic acid	<0.006
L-Histidine	<0.005
Tryptamine	<0.005
Tyramine	<0.005

Recovery

Increasing amounts of 1-methylhistamine were added to an urine sample, to a plasma sample and to two cell culture media. Each spiked sample was assayed. The analytical recovery of 1-methylhistamine was estimated at different concentrations by using the theoretically expected and the actually measured values.

Concentrations in ng/ml

Urine

Added	Measured	Expected	% recovery
0	18.6		
9.7	32.1	28.3	114
18.8	40.0	37.4	107
38.5	73.7	57.1	129
56.6	93.7	75.2	125
74.1	110.0	92.7	119
90.9	96.8	109.5	88
143	156.1	161.5	97
188	174.4	206.1	85



231	274.5	249.4	110
273	291.7	291.3	100
385	433.7	403.2	108
476	542.7	494.8	110
566	647.8	584.6	111

Mean Recovery: 108

Plasma

Added	Measured	Expected	% recovery
0.00	0.55		
0.29	0.77	0.84	92
0.74	1.29	1.29	100
1.43	2.14	1.98	108
2.31	2.87	2.86	101
4.76	5.05	5.31	95
9.09	7.99	9.64	83

Mean recovery: 96

DMEM

Added	Measured	Expected	% recovery
0.00	0.66		
0.29	0.83	0.95	87
0.74	1.38	1.40	99
1.43	2.47	2.09	118
2.31	3.30	2.97	111
4.76	6.20	5.42	114
9.09	11.05	9.75	113

Mean recovery: 107

RPMI

Added	Measured	Expected	% recovery
0.00	0.53		
0.29	0.81	0.82	98
0.74	1.41	1.27	110
1.43	2.35	1.96	120
2.31	3.31	2.84	116
4.76	5.58	5.29	105
9.09	11.61	9.62	121

Mean recovery: 112

Linearity

The linearity of the ELISA method was investigated using different dilutions of an urine sample (diluent: CAL 1).
Concentrations in ng/ml



dilution	measured	recalculated value	recovery %
orig.	241.4		
4+1	204.4	193.1	106
3+1	188.6	181.5	104
2+1	158.0	160.9	98
1+1	137.2	120.7	114
1+2	82.1	80.5	102
1+3	66.2	60.3	110
1+4	49.6	48.3	103
1+5	41.2	40.2	103
1+7	32.8	30.2	109
1+9	26.9	24.1	111
mean linearity:		106	

Reproducibility

The reproducibility of the ELISA method was investigated determining the intra-assay-coefficients of variation (cv) by repeated measurements of two urine samples, one EDTA-plasma sample and one cell culture sample (RPMI) with different 1-methylhistamine concentrations.

Concentrations in ng/ml

Sample	N	Mean value	Sd	CV (%)
Urine 1	40	33.97	2.07	6.1
Urine 2	40	113.95	8.68	7.3

Sample	N	Mean value	Sd	CV (%)
Plasma	40	0.957	0.087	9.7

Sample	N	Mean value	Sd	CV (%)
RPMI	40	0.990	0.070	7.1

Correlation 1-Methylhistamine by ELISA to LCMA

Correlation to LCMS:

$$Y = 0.94 \times \text{LCMS} + 6.5 \quad R = 0.968 \quad N = 16$$



Preparation of Urine Samples (Acylation)

		Standard	Control	Urine Sample
Standard 1 - 6	µl	20		
Control 1 & 2	µl		20	
Urine Sample	µl			20
Equalizing Reagent	µl	200	200	200
Freshly prepared Acylation Reagent	µl	20	20	20

Immediately: Shake for 60 minutes at room temperature
Do not cover wells or plate, leave the plate open on the shaker

Take each 20 µl for the ELISA

ELISA Urine Samples

		Standard	Control	Urine Sample
Start Buffer	µl	50	50	50
Acyl. Standard 1 - 6	µl	20		
Acyl. Control 1 & 2	µl		20	
Acyl. Urine Sample	µl			20
Antiserum	µl	50	50	50

Shake for 30 minutes at room temperature
Do not cover wells or plate, leave the plate open on the shaker

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 15 – 20 minutes at room temperature

Stop Solution	µl	100	100	100
---------------	----	-----	-----	-----

Reading of absorbance at 450 nm



Pipetting Scheme Plasma and Cell Culture Samples

Preparation of Plasma and Cell Culture Samples (Acylation)

	Standard	Control	Sample
Standard 1 – 6 (1:50) μ l	50		
Control 1 & 2 (1:50) μ l		50	
Sample μ l			50
Equalizing Reagent μ l	100	100	100
Freshly prepared Acylation Reagent μ l	10	10	10

Immediately: Shake for 60 minutes at room temperature
Do not cover wells or plate, leave the plate open on the shaker

Take each 50 μ l for the ELISA

ELISA Plasma and Cell Culture Samples

	Standard	Control	Sample
Start buffer μ l	50	50	50
Acyl. Standard 1 - 6 μ l	50		
Acyl. Control 1 & 2 μ l		50	
Acyl. Sample μ l			50
Antiserum μ l	20	20	20

Cover the plate with adhesive foil
Shake plate briefly
Incubate for 15 – 20 hours (overnight) at 2 – 8 °C

4 x washing

Enzyme Conjugate μ l	100	100	100
--------------------------	-----	-----	-----

Shake for 20 minutes at room temperature

4 x washing

Substrate μ l	100	100	100
-------------------	-----	-----	-----

Shake for 15 – 20 minutes at room temperature

Stop Solution μ l	100	100	100
-----------------------	-----	-----	-----

Reading of absorbance at 450 nm



Warranty Information

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

Eagle Biosciences makes no warranties, either expressed or implied, except as provided herein, including without limitation thereof, warranties as to marketability, merchantability, fitness for a particular purpose or use, or non-infringement of any intellectual property rights. In no event shall the company be liable for any indirect, incidental, or consequential damages of any nature, or losses or expenses resulting from any defective product or the use of any product. Product(s) may not be resold, modified, or altered for resale without prior written approval from Eagle Biosciences, Inc.

For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.