Histamine ELISA

Catalog No. E-HIS-K65

IVD CE

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ENZYME IMMUNOASSAY FOR THE IN VITRO DETERMINATION OF HISTAMINE IN BIOLOGICAL SAMPLES

1. PRINCIPLE OF THE ASSAY

First, Histamine is quantitatively acylated. The subsequent competitive ELISA kit uses the microtiter plate format. The antigen is bound to the solid phase of the microtiter plate. The acylated standards, controls and samples and the solid phase bound analyte compete for a fixed number of antiserum binding sites. After the system is in equilibrium, free antigen and free antigen-antiserum complexes are removed by washing. The antibody bound to the solid phase is detected by an anti-rabbit IgG-peroxidase conjugate using TMB as a substrate. The reaction is monitored at 450 nm.

Quantification of unknown samples is achieved by comparing their absorbance with a reference curve prepared with known standard concentrations.

2. REAGENTS PROVIDED

Before opening, all reagents of the kit are stable until the expiry date indicated on the kit labels, if stored at 2-8°C.

After opening, store the vials one month at 2-8°C.

REF	LABEL	COMPONENT	VOLUME	REMARK
E-PR-60	PR MTP	**Preperation Microtiter Plate**	1 x 96 wells	ready for use
E-WAC-30	WAS C 10x	**Wash Concentrate 10x concentrated**	1 x 100 mL	concentrate, dilute content with ultrapure water to a final volume of 1000 mL
E-TMB-08	TMB SUB	**TMB-Substrate**	1 x 12 mL	ready for use, containing a solution of tetramethylbenzidine (TMB)
E-STP-09	STP	**Stopping Solution**	1 x 12 mL	ready for use, containing 0.25 M H_2SO_4.
E-HIS-24	ACY BUF	**Acylation Buffer**	2 x 3 mL	ready for use
E-HIS-10	HIS MTP	**Histamine Microtiter Plate**	1 x 96 wells	12 strips, 8 wells each, break apart, precoated. Unused strips may be stored at 2-8°C in the self-lock bag provided.
E-HIS-20	CONJ	**Conjugate**	1 x 12 ml	ready for use, anti-rabbit IgG conjugated with peroxidase
E-HIS-01	CAL 0	**Calibrator 0**	1 x 2 mL	ready for use
E-HIS-02	CAL 1	**Calibrator 1**	1 x 2 mL	ready for use
E-HIS-03	CAL 2	**Calibrator 2**	1 x 2 mL	ready for use
E-HIS-04	CAL 3	**Calibrator 3**	1 x 2 mL	ready for use
E-HIS-05	CAL 4	**Calibrator 4**	1 x 2 mL	ready for use
E-HIS-05	CAL 5	**Calibrator 5**	1 x 2 mL	ready for use
E-HIS-25	HIS AB	**Histamine Antibody**	1 x 12 mL	from goat, ready for use
E-HIS-35	ACY REA	**Acylation Reagent**	1 x 3 mL	ready for use
E-HIS-07	CONT L	**Control Low**	1 x 2 mL	ready for use
E-HIS-08	CONT H	**Control High**	1 x 2 mL	ready for use

3. MATERIAL REQUIRED BUT NOT PROVIDED

In addition to standard laboratory equipment, the following items are required: - For the immunoassay:

- plastic tubes (3 or 5 mL)
- precision micropipets (10-100, 100-1000 µL) - repeating micropipets (25, 100 and 300 µL)
- "vortex" type mixer
- microtiter plate shaker washer (optional)
- microtiter plate reader (450 nm)
- distilled water

4. PRECAUTIONS

4.1 General remarks:

- Bring all reagents to room temperature before pipeting.
- Do not mix the reagents from kits of different lots.
- A standard curve must be included with each assay.
- It is recommended to perform the assay in duplicate.
- Gloves must be worm to avoid contamination by extraneous histamine, e. g. from perspiration.
- Histamine adsorbs on glass, use only plastic pipets, tubes etc.

5. SPECIMEN COLLECTION, PROCESSING AND STORAGE

Waste should be discarded according to the country rules.

5.1 Plasma

- collect blood into a chilled tube containing EDTA only and chill immediately on ice.
- centrifuge 10 minutes at 900 g at 4°C within 20 minutes of sample collection. - aspirate the upper 2/3 of plasma.
- plasma may be diluted in Diluent (supply upon request).

5.2 Urine

- collect urine specimen in plastic container. Either freeze sample or add bacteriostatic agent.
- dilute samples 1:5 in distilled water.

5.3 Histamine release in whole blood

Do not vortex cell suspension.

Blood sampling: collect 1-2 mL of blood into heparine tubes only, do not use EDTA. Keep samples at 18-25°C for a maximum of 24 hours if the assay cannot be done immediately.

Total histamine after cell lysis: Add 50 µL of undiluted blood to 950 µL of distilled water (dilution 1:20). Freeze and thaw twice.

5.4 Liquid samples

Collect samples in plastic tubes.

5.5 Sample storage

Except for cell challenge whole blood, store all type of sample at <-18°C in plastic tubes if the assay cannot be done immediately.

6. ASSAY PROCEDURE

6.1 Preparation of reagents

- Let the reagents come to room temperature.
- Pour the content of the wash solution concentrated vial into 980 mL of distilled water and homogenize.

6.2 Preparation of samples: acylation

The assay procedure includes an acylation step for samples, calibrators or controls. The acylated samples are quite stable and may be stored 7 days at 2-8°C.

6.3 Assay procedure

Acylation step	Immunological step	Immunological step	Enzymatic step	Reading
To *Reaction Microtiter Plate*, add *50 µL* of *Acylation Buffer*, *25 µL* of *Calibrator, control* or *sample*, *25 µL* of *Acylation Reagent* and incubate 30 minutes at 37°C	To *Histamine Microtiter Plate* add *25 µL* of *acylated sample*, *control* or *calibrator* and 100 µL of *Histamine Antibody* and incubate 30 minutes at 37°C	Discard or aspirate the contents of the wells and wash each well 4 times thoroughly with *300 µL diluted wash solution** add 100 µL of *HRP Conjugate* and incubate 30 minutes at 37°C	Discard or aspirate the contents of the wells and wash each well 4x times thoroughly with *300 µL diluted wash solution** Add 100 µL of *Substrate* and incubate 15-20 minutes at RT (20-25°C)	add *100 µL* of *Stop Solution*, Read absorbance at 450 nm

** Plate washing: This step is essential to obtain the expected kit performance. After washing, wells must not dry prior to the addition of the next reagent.*

Using a microtiter plate washer

Select a three cycle plate washing program that meets the following cycle criteria :
Fluid in wells must be completely aspirated
Wells must be filled to the rim with the wash solution
Wash solution must be injected rapidly (typically one second to fill one well)
After the three cycles, wash solution must be completely aspirated

Manuall procedure

Repeat at least three cycles as follows:
Turn plate upside-down and shake vigorously over the sink
Fill wells with wash solution, the solution may run over the rim of the wells
Turn plate upside-down and shake vigorously over the sink, and firmly tap the inverted microtiter plate onto a clean absorbent paper

7. RESULTS

Results are obtained from the standard curve by interpolation. The curve serves for the determination of histamine concentration in samples measured at the same time as the calibrator.

7.1 Standard curve

The results in the package insert were calculated using a semi-logarithmic curve fit ("logistic" mode) with absorbance values on vertical axis and the histamine concentration of the calibrators on the horizontal axis (nM). Other data reduction methods may give slightly different results.

Calibrator	Histamine (nM)	Histamine (ng/mL)	Absorbance
0	0	0	2.212
1	2.5	0.25	1,998
2	10	1	1.592
3	40	4	1.063
4	160	16	0.614
4	640	64	0.257
(Example of standard curve, do not use for calculation)			

7.2 Samples

- Plasma concentrations correspond to values read off the standard curve
- Urine concentrations must be multiplied by 5.
- Values obtained for total histamine must be multiplied by 20.
- Other samples results must be corrected for the proper dilution factor.

7.2 Controls

Control	Histamine (nM)	Histamine (ng/mL)
Low	3 - 9	0.3 – 0.9
High	5	0.5

8. QUALITY CONTROL

Good laboratory practices imply that control samples be used regularly to ensure the quality of the results obtained. These samples must be processed exactly the same way as the assay samples, and it is recommended that their results be analysed using appropriate statistical methods.

In case of packaging deterioration or if data obtained show some performance alteration, please contact your local distributor or the manufacturer.

9. EXPECTED VALUES

We recommend each laboratory to establish its own reference values. The following values obtained from healthy subjects are indicative only.

- Plasma: < 10 nM (1 ng/mL)
- Whole blood: 200-2000 nM (20-200 ng/mL)
- Urine: 10-35 µg/g of creatinin

10. PERFORMANCE CHARACTERISTICS

10.1 Analytical sensitivity: 0.5 nM

10.2 Specificity:

Analytical Specificity (Cross Reactivity)	Substance	Cross Reactivity (%)
	acylated histamine	100
	acylated 3 methylhistamine	< 0.1
	acylated 1 methylhistamine N-acetylimidazole acylated serotonin acylated histidine	< 0.001

10.3 Precision

Intra-assay:
Samples were assayed in 20 times in the same serie. The coefficients of variation were found below or equal to 9.9 %.

Inter-assay:
Samples were assayed in duplicate in 10 different series. Coefficients of variation were found below or equal to 12.0 %.

10.4 Accuracy

Dilution test
High-concentration samples were serially diluted 1:16 with the distilled water. The recovery percentages obtained were between 90% and 125%.

Recovery test
Low-concentration samples were spiked with know quantities of histamine. The recovery percentages obtained were between 85% and 119%.

10.5 Measurement range (from analytical sensitivity to highest calibrator): 1 – 512 nM.

11. LIMITATIONS OF THE PROCEDURE

The non-respect of the instructions in this package insert may affect results significantly. Results should be interpreted in the light of the total clinical presentation of the patient, including clinical history, data from additional tests and other appropriate information.
Do not use hemolyzed or icteric samples.

Interferences
- Hemolysed plasma must not be used.
- Pregnant women blood contains high levels of diamine oxydase and cannot be used.
- Material containing amine other than histamine at a concentration in excess of 10 mM interferes with the acylation of histamine.
- Phenol inhibits histamine release.
- Histamine may be a component of allergen such as hymenopteran venom.

12. SYMBOLS

	Use by / Utiliser jusqu'à / Verwendbar bis / Utilizzare entro / Use antes de / ΗμΕΡΟμΗΝΊΑ ΛΉΞΗΣ / Použitelné do / PoužiteĽné do / UŽyĆ do / Felhasználható/ Naudoti iki / ИСПОЛЬЗОВАТЬ ДО
	In Vitro diagnostic Device / Diagnostic in Vitro / InVitro Diagnostikum / Diagnostico in vitro/ Diagnostico in Vitro / In Vitro ΔΙΑΓΝΩΣΤΙΚΌ ΙΑΤΡΟΤΕΧΝΟΛΟΓΙΚΌ ΠΡΟΪΟΝú / Pro diagnostiku in vitro / Pre diagnostiku in vitro / Do diagnostyki in vitro / In vitro diagnosztikum / In vitro diagnostikai / ДЛЯ ИН ВИТРО ДИАГНОСТИКИ
	Catalogue Number / Référence catalogue / Bestellnummer / Numero di catalogo / Numero de catalogo / ΑΡΙΘμΌΣ ΚΑΤΑΛΌΓΟΥ / Katalogové Číslo / Katalógové Číslo / Numer katalogowy / Katalógusszám / Katalogo Nr. / КАТАЛОЖНЫЙ №
	Batch code / Numéro de lot / Chargenbezeichnung / Coddice del lotto / Codigo de lote / ΑΡΙΘμΌΣ ΠΑΡΤΊΔΑΣ / Číslo šarže / Číslo šarže / Numer serii / Lot szám / Serijos Nr. / № СЕРИИ
	Caution, see instructions for use / Attention, consulter la notice d'utilisation / Achtung, Gebrauchsanweisung beachten / Attenzione vedere le istruzioni per l'uso / ΠΡΟΕΙΔΟΠΟΊΗΣΗ, ΣΥμΒΟΥΛΕΥΤΕΊΤΕ ΤΑ ΣΥΝΟΔΆ ΈΝΤΥΠΑ / Pozor, ČtĚte pozornĚ návod / Pozor, Čítajte návod pozorne / Uwaga, patrz instrukcja uŻycia / Figyelem, olvassa el a használati utasítást / DĖmesio, atidžiai perskaitykite instrukcijĄ / ВНИМАНИЕ, ЧИТАЙТЕ ТЩАТЕЛЬНО ИНСТРУКЦИЮ
	Consult Instructions for Use / Consulter la notice d'utilisation / Gebrauchsanweisung beachten / Leggere le istruzioni per l'uso / Consulte las instrucciones de uso / ΣΥμΒΟΥΛΕΥΤΕΊΤΕ ΤΙΣ ΟΔΗΓΊΕΣ ΧΡΉΣΗΣ / Viz návod / ViĎ návod / Patrz instrukcja uŻycia / Olvassa el a használati utasítást / ŽiŪrĖti instrukcijĄ / СМОТРИ ИНСТРУКЦИЮ
	Temperature limitation / Limites de température / Temperaturgrenzen / Limiti di temperatura / Limites de temperatura / ΠΕΡΙΟΡΙΣμΟΊ ΘΕΡμΟΚΡΑΣΊΑΣ / Rozmezí skladovacích teplot / Rozsahy skladovacích teplôt / Ograniczenie temperatury / HŐmérséklethatárok / Saugojimo temperatŪrŲ intervalas / ДИАПАЗОН ТЕМПЕРАТУР ХРАНЕНИЯ
	For XX tests / Pour XX dosages / Für XX Bestimmungen / Per XX dosaggi / Para XX ensayos / ΠΕΡΙΕΧΌμΕΝΟ ΕΠΑΡΚΈΣ ΓΙΑ ΧΧ ΕΞΕΤΆΣΕΙΣ / Lze použít pro XX testŮ / UrČené na XX testov / ZawartoŚĆ na XX testów / Tartalma XX teszt elvégzésére elegendŐ / Pakanka XX testui atlikti / ПРЕДНАЗНАЧЕН ДЛЯ XX ТЕСТОВ
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