Human Histamine ELISA Kit

Enzyme Immunoassay for the quantification of Histamine in plasma and urine samples.

Catalog number: ARG80455

Package: 96wells

TABLE OF CONTENTS

MANUFACTURED BY:

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PRINCIPLE OF THE ASSAY

This is an Enzyme Immunoassay for the quantification Histamine in plasma and urine samples.

This assay employs the competitive quantitative enzyme immunoassay technique. Histamine is first quantitatively acylated.

The antigen has been pre-coated onto a microtiter plate. Acylated controls, standards or samples and the solid phase bound analytes 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. Anti-goat IgG conjugated to Peroxidase is added to each well and incubated. After washing away any unbound antibody conjugate, a substrate solution (TMB) is added to the wells and color develops in inverse-proportion to the amount of Histamine present in the samples. The color development is stopped by the addition of STOP solution and the intensity of the color is measured at a wavelength of 450nm ±2nm. The concentration of Histamine in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Histamine-coated microplate	12 strips X 8 wells	4°C
Adhesive foil	4 pieces	RT
50X Wash Buffer	20ml	4°C

Anti-goat IgG-peroxidase conjugate	12ml (Ready-to-use)	4°C
TMB substrate	12ml (Ready-to-use)	4°C (Protect from light)
STOP solution	12ml (Ready-to-use)	4°C
Standard A-F	4ml each (Ready-to-use)	4°C
Histamine Antiserum	6ml (Ready-to-use)	4°C
Acylation Buffer	4ml (Ready-to-use)	4°C
Acylation Reagent	4 X 1.25ml (Lyophilized)	4°C
Acylation Diluent	2 X 4ml (Ready-to-use)	4°C
Reaction Plate	1 X 96 wells (Ready-to-use)	4°C
Control 1	4ml (Ready-to-use)	4°C
Control 2	4ml (Ready-to-use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Briefly spin down all vials before use.
- If crystals are observed in the 50X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.

- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Do not use haemolytic or lipemic samples. Assay immediately (up to 6 hours at 2-8 °C), or aliquot and store samples at ≤ -20 °C (up to 6 months). Avoid repeated freeze-thaw cycles. Avoid exposure to direct sunlight.

Urine – Spontaneous or 24-hour urine, collected in a bottle containing 10-15ml of 6M HCl. For linger storage, aliquot and store samples at ≤ -20 °C (up to 6 months). Avoid repeated freeze-thaw cycles. Avoid exposure to direct sunlight.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 50X Wash buffer into distilled water to yield 1X Wash buffer. Storage: up to 6 months at 4-8°C.
- Acylation diluent:The Acylation Diluent has a freezing point of 18.5 °C. Before use, it must be ensured that the acylation diluent has reached room temperature and form a homogenous, crystal-free solution. Alternatively, the acylation diluent can be stored at RT separated from other components form the kit.
- Acylation Reagent: Reconstitute each vial with 1.25 ml acylation diluent. Prepare fresh prior to use. (Not longer than 1 hour in advance).

ASSAY PROCEDURE

Sample Preparation and Acylation

1. Pipette 25µl of standards, controls, plasma samples and 10µl of urine samples into the appropriate wells of the Reaction Plate.
2. Add 25µl Acylation Buffer into all wells.
3. Add 25µl Acylation Reagent into all wells.
4. Incubate for 45 mins at RT on shaker (~600rpm)
5. Add 200µl Distilled water into all wells.
6. Incubate for 15 mins at RT on shaker (~600rpm)
7. Use 25µl for Histamine assay.

Histamine ELISA procedure

1. Remove excess microtiter strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 25 µl of the acylated standards, controls and samples into the appropriate wells of Histamine Microtiter Strips.
3. Add 100 µl of Histamine Antiserum into wells.
4. Cover plate with Adhesive foil and incubate for 3 hours at RT on a shaker (600rpm). (Alternatively, incubate for 15-20 hours at 2-8°C.)
5. Remove the foil and discard. Aspirate each well and wash, repeating the process 4 times for a total 5 washes. Wash by filling each well with 1× Wash Buffer (350 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by

aspirating, decanting or blotting against clean paper towels.

6. Add 100 µl of Anti-rabbit IgG-peroxidase conjugate into wells.
7. Incubate for 30 mins at RT on a shaker (600rpm).
8. Aspirate each well and wash as step 5.
9. Add 100 µl of TMB substrate solution into each well. Incubate for 20-30 mins at RT with shaking (600rpm). Avoid exposure to light.
10. Add 100 µl of Stop Solution to each well and shake lightly to ensure homogeneous mixing.
11. Read the OD with a microplate reader at 450nm (with a reference wavelength between 620nm and 650nm) within 10 minutes.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and samples.

2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

5. The concentrations of undiluted samples and controls can be read directly

from the standard curve.

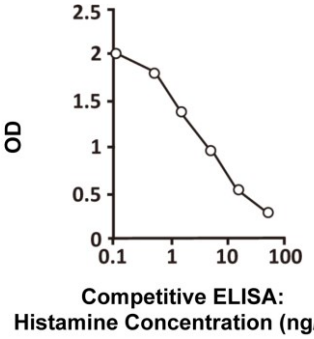
6. Refer to the table below for molar conversion:

	Concentration of standards					
Standard	A	B	C	D	E	F
Histamine (ng/ml)	0	0.5	1.5	5	15	50
Histamine (nmol/L)	0	4.5	13.5	45	135	450
Conversion	Histamine (ng/ml) x 9 = Histamine (nmol/L)					

- Urine samples: The read concentrations have to be multiplied by 2.5. Calculate the 24h excretion for each urine sample: µg/24h = µg/L X L/24h
- Plasma samples and controls: The concentration of can be read directly from the standard curve.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

urine: 0.3 ng/ml; plasma: 0.12 ng/ml

Assay Range

0.5-50 ng/ml

Specificity

No significant cross-reactivity was found for the following factors:

3-Methyl Histamine, Tyramine, L-Phenylalanine, L-Histidine, L-Tyrosine, Tryptamine, 5-Hydroxy-Indole-Acetic Acid, Serotonin

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 7.3% (urine) and 6.8% (plasma) and inter-assay precision was 9.15%.

Recovery

108-123% (Histamine-urine), 92-120% (Histamine-plasma)

Linearity

90-124% (Histamine-urine), 85-106% (Histamine-plasma)