Histamine Research ELISA Kit

Enzyme Immunoassay for the quantification of Histamine in EDTA-Plasma and other biological samples types form different animal species.

Catalog number: ARG80459

Package: 96 wells

TABLE OF CONTENTS

SECTION **Page**

PRINCIPLE OF THE ASSAY 3
MATERIALS PROVIDED & STORAGE INFORMATION 3
MATERIALS REQUIRED BUT NOT PROVIDED 4
TECHNICAL HINTS AND PRECAUTIONS 4
SAMPLE COLLECTION & STORAGE INFORMATION 5
REAGENT PREPARATION 5
ASSAY PROCEDURE 6
CALCULATION OF RESULTS 7
EXAMPLE OF TYPICAL STANDARD CURVE 8
QUALITY ASSURANCE 9

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PRINCIPLE OF THE ASSAY

This is an Enzyme Immunoassay for the quantification of Histamine in EDTA-Plasma and other biological samples types form different animal species. For samples from cell culture supernatants, we recommended ARG80457 Human Histamine ELISA Kit for Histamine detection.

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
Diluent	22ml (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	12ml (Ready-to-use)	4°C
Acylation Buffer	4ml (Ready-to-use)	4°C
Acylation Reagent	4 X 2ml (Lyophilized)	4°C
Acylation Diluent	10ml (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

Collect samples using EDTA 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.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 50X Wash buffer into distilled water to yield 1X Wash buffer. Storage: up to 6 months at 2-8°C.
- Acylation Reagent: Reconstitute each vial with 2 ml acylation diluent. Prepare fresh prior to use. (Not longer than 1 hour in advance). If more than 2 ml are needed, pool the content of the individual vials and mix thoroughly.

ASSAY PROCEDURE

Sample Preparation and Acylation

1. **Samples dilution:** Dilute sample 1: 21 (for example, 10µl sample + 200µl diluent). Vortex for 1 min at RT.
2. Pipette 25µl of standards, controls and diluted samples into the appropriate wells of the Reaction Plate.
3. Add 25µl Acylation Buffer into all wells.
4. Add 25µl Acylation Reagent into all wells.
5. Incubate for 45 mins at RT on shaker (~600rpm)
6. Add 100µl Distilled water into all wells.
7. Incubate for 15 mins at RT on shaker (~600rpm)
8. Use 25µl acylated standards, controls and samples 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 25µl 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. Shake briefly and incubate for 20-25 hours at 2-8°C (with foil cover). (or incubate for 3 hours at RT (20-25°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-goat 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. (In case of overflow, read the OD at 405nm 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.

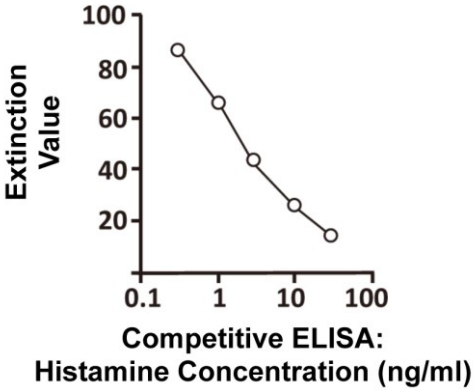
6. Refer to the table below for molar conversion:

	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)					

- Controls: The concentrations can be read directly from the standard curve
- Samples: The concentrations of samples have to be multiplied by 21.

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

0.2 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