

ELISA kits available from ADI (see details at the web site)

Catalog# ProdDescription

1960-10 Epinephrine/Adrenaline (High Sensitivity) ELISA Kit, 96 tests, Quantitative
 1960-20 Norepinephrine/Noradrenaline (High Sensitivity) ELISA Kit, 96 tests, Quantitative
 1960-30 Histamine (High Sensitivity) ELISA Kit, 96 tests, Quantitative

#0010	Human Leptin		
#200-120-AGH	Human globular Adiponectin (gAcrp30)		
#0700	Human Sex Hormone Binding Glob (SHBG)		
#0900	Human IGF-Binding Protein 1 (IGFBP1)		
#1000	Human C-Reactive Protein (CRP)		
#100-110-RSH	Human Resistin /FIZZ3		
#100-140-ADH	Human Adiponectin (Acrp30)		
#100-160-ANH	Human Angiogenin		
#100-180-APH	Human Angiopoietin-2 (Ang-2)		
#100-190-B7H	Human Bone Morphogenic Protein 7 (BMP-7)		
#1190	Human Serum Albumin	#1200	Human Albumin (Urinary)
#1750	Human IgG (total)	#1760	Human IgM
#1800	Human IgE	#1810	Human Ferritin
#1210	Human Transferrin (Tf)	#0020	Beta-2 microglobulin
#1600	Human Growth Hormone (GH)		
#0060	Human Pancreatic Colorectal cancer (CA-242)		
#1820	Human Ovarian Cancer (CA125)	#1830	Human CA153
#1840	Human Pancreatic & GI Cancer (CA199)		
#1310	Human Pancreatic Lipase		
#1400	Human Prostatic Acid Phosphatase (PAP)		
#1500	Human Prostate Specific Antigen (PSA)	#1510	free PSA (fPSA)
#0500	Human Alpha Fetoprotein (AFP)		
#0050	Human Neuron Specific Enolase (NSE)		
#0030	Human Insulin	#0040	Human C-peptide
#0100	Human Luteinizing Hormone (LH)		
#0200	Human Follicle Stimulating Hormone (FSH)		
#0300	Human Prolactin (PRL)		
#0400	Human Chorionic Gonadotropin (HCG)	#0410	HCG-free beta
#0600	Human Thyroid Stimulating Hormone (TSH)		
#1100	Human Total Thyroxine (T4)	#1110	Human Free T4 (fT4)
#1650	Human free triiodothyronine (fT3)	#1700	Human T3 (total)
#1850	Human Cortisol	#1860	Human Progesterone
#1865	Human Pregnenolone	#1875	Human Aldosterone
#1880	Human Testosterone	#1885	Human free Testosterone
#1910	Human Androstenedione	#1920	Human Estradiol
#1925	Human Estrone	#1940	Dihydrotestosterone (DHT)
#1950	Human DHEA-sulphate (DHEA-S)		
#3400	Human serum Neopterin		
#3000	Human Rheumatoid Factors IgM (RF)		
#3100	Human anti-dsDNA		
#3200	Anti-Nuclear Antibodies (ANA)		

Instruction Manual No. M-1960-30

Histamine ELISA kit

ELISA KIT Cat. No. 1960-30, 96 Tests

For Quantitative Determination of Histamine in serum, plasma, cell culture, whole blood or urine.



For In Vitro Research Use Only


ALPHA DIAGNOSTIC
INTERNATIONAL

India Contact:

Life Technologies (India) Pvt. Ltd.

306, Aggarwal City Mall, Opposite M2K Pitampura, Delhi – 110034
 Ph: +91-11-42208000, 42208111, 42208222

Mobile: +91-9810521400

Fax: +91-11-42208444

Email: customerservice@atzlabs.com

Web: www.atzlabs.com


Life Technologies™
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Histamine ELISA KIT # 1960-30

ELISA Kit for the quantitative determination of Histamine in plasma. For in vitro diagnostic use only. : [Kit Contents \(96 tests\)](#):

Components	Cat. No.
Reaction Plate , 96 wells	196031
Adhesive Foils, Qty:4	19603011F
Wash buffer (50X) , 20 ml	196030-WB
Substrate Solution , 12 ml	196030-EC
Stop Solution , 12 ml	196030-SU
Histamine Stds. A-F (0, 0.5, 1.5, 5, 15, 50 ng/ml) 6 vials x 4 ml each	196032A-F
Histamine Antiserum , 12 ml	196030-14
Acylation Buffer , 4 ml	196030-AB
Acylation Reagent , 2 X 2 ml Lyophilized	196030-AR
Histamine microtiter strips (96 wells, 12 x 8 wells)	196030-15
Enzyme Conjugate (anti-goat IgG-HRP) 1x12 ml	196030-16
Histamine Control 1 (4 ml)	196030-C1
Histamine Control 2 (4 ml)	196030-C2
Acylation diluent , 5 ml	196030-AD
Complete Instruction Manual	M-1960-30

Intended Use

Enzyme Immunoassay for the determination of Histamine in plasma and urine. For research use only, not for use in diagnostic procedures (RUO).

General Information

Histamine is an organic nitrogenous compound involved in local immune responses as well as regulating physiological function in the gut and acting as a neurotransmitter. Histamine is involved in the inflammatory response. As part of an immune response to foreign pathogens, histamine is produced by basophils and by mast cells found in nearby connective tissues. Histamine increases the permeability of the capillaries to white blood cells and some proteins, to allow them to engage pathogens in the infected tissues. Histamine is a chemical messenger and aminergic neurotransmitters, playing an important role in a multitude of physiological processes in central and peripheral tissues. Histamine is synthesized in a restricted population of neurons located in the tuberomammillary nucleus of the posterior hypothalamus implicated in many brain functions (e.g. sleep/wakefulness, hormonal secretion, cardiovascular control, thermoregulation, food intake, and memory formation). In peripheral tissues histamine is stored in mast cells, basophils, enterochromaffin cells. Mast cell histamine plays an important role in the pathogenesis of various allergic conditions. Histamine release leads to various well-known symptoms of allergic conditions in the skin and the airway system. Histamine-mediated effects are mediated through four pharmacologically distinct subtypes of receptors, i.e., the H1, H2, H3 and H4 receptors, which are all members of the G-protein coupled receptor (GPCR) family. Histamine receptors display 7 TM domains, an extracellular N-terminus, and a cytoplasmic C-terminus of variable length.

Precisions:

Intra-Assay	Sample	Range (ng/mL)	CV (%)
Histamine Urine	1	8.7 ± 0.6	7.4
	2	30.1 ± 2.2	7.3
Histamine Plasma	1	2.03 ± 0.16	8
	2	6.74 ± 0.37	5.6

Inter-Assay	Sample	Range (ng/mL)	CV (%)
Histamine Control samples	1	0.6 ± 0.1	12
	2	4.6 ± 0.3	6.3

Linearity:

		Range	Serial dilutions up to	Range (%)
Histamine	Urine	4.33 - 70 ng/mL	1:16	90 - 124
	Plasma	0.74 - 8.48 ng/mL	1:16	85 -106

Recovery:

		Mean (%)	Range	% Recovery after spiking
Histamine	Urine	112	108- 123	
	Plasma	103	92- 120	

Method comparison versus ELISA

Histamine

Urine ELISA = 0.9 ELISA (ADI) – 3.1 $r = 0.98$; $n = 29$

Plasma ELISA = 1.0 ELISA (ADI) – 0.4 $r = 0.99$; $n = 47$

Collection and Handling of Unknowns:

Plasma

Plasma (EDTA, Heparin) should be used. Haemolytic and especially lipemic unknowns should not be used with this assay. Storage: up to 6 hours at 2 - 8°C; for longer periods (up to 6 months) at - 20°C. Repeated freezing and thawing should be avoided.

Urine:

Spontaneous or 24-hour urine, collected in a bottle containing 10-15 mL of 6 M HCl, may be used. Storage: up to 6 hours at 2 - 8°C; for longer periods (up to 6 months) at -20°C. Repeated freezing and thawing should be avoided. Avoid exposure to direct sunlight.

Whole Blood:

The histamine release is performed with heparinized whole blood. For further information please refer to the instructions for use of the Histamine elisa.

Plasma unknowns and controls:

The concentrations of the plasma unknowns and the controls can be read directly from the calibration curve.

Urine unknowns:

The read concentrations of histamine in urine have to be multiplied by 2.5

The total amount of Histamine excreted in urine during 24 h is calculated as following: $\mu\text{g}/24\text{h} = \mu\text{g}/\text{L} \times \text{L}/24\text{h}$

Quality control

It is recommended to use controls according to state and federal regulations. The kit, or other commercially available, controls should fall within established confidence limits. The confidence limits of the kit controls are printed on the QC-Report.

Calibration

The binding of the antisera and the enzyme conjugates and the activity of the enzyme used are temperature dependent, and the extinction values may vary if a thermostat is not used. The higher the temperature, the higher the extinction values will be. The extinction values also depend on the incubation times. The optimal temperature during the Enzyme Immunoassay is between 20-25°C.

In case of overflow, read the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to 405 nm

Analytical Specificity (Cross Reactivity)

Substance	Cross Reactivity (%)
Histamine	100
3-Methyl-Histamine	0.1
Tyramine	0.01
L-Phenylalanine	< 0.001
L-Histidine	< 0.001
L-Tyrosine	< 0.001
Tryptamine	< 0.001
5-Hydroxy-Indole-Acetic Acid	< 0.001
Serotonin	< 0.001

Analytical Sensitivity (Limit of detection):

Histamine	
Sensitivity Plasma	0.12 ng/mL
Sensitivity Urine	0.30 ng/mL

PRINCIPLE OF THE TEST

The assay can be used for the determination of histamine release in heparinized whole blood. In the first part of the procedure, Histamine is 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 calibrators, controls and unknowns 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. Determination of unknowns is achieved by comparing their absorbance with a reference curve prepared with known calibrator concentrations.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (5-1000 μl) and multichannel pipet with disposable plastic tips. Reagent troughs, plate washer (recommended) and ELISA plate Reader.

PRECAUTIONS

The ADI's ELISA test is intended for *in vitro research* use only. The reagents contain thimerosal as preservative; necessary care should be taken when disposing solutions. The Endpoint Cutoff and Positive controls have been prepared from human sera shown to be negative for HBsAg and HIV antibodies. Nevertheless, such tests are unable to prove the complete absence of viruses, therefore, sera should be handled with appropriate precautions.

Applicable **MSDS**, if not already on file, for the following reagents can be obtained from ADI or the web site. TMB (substrate), H₂SO₄ (stop solution), and Proclin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates).

All waste material should be properly disinfected before disposal. Avoid contact with the stop solution (1N sulfuric acid).

SAMPLE COLLECTION AND HANDLING

EDTA-Plasma should be used. Do not use haemolytic or lipemic samples. Storage: up to 6 hours at 2 - 8°C; for longer periods (up to 6 months) at - 20°C. Repeated freezing and thawing should be avoided.. Avoid repeated freezing and thawing of samples. No preservatives should be added to the serum.

Preparation of the reagent:

Dilute wash buffer (1:50) with distilled water (20 ml stock in total of 1-liter). store at 4oC.

Acylation Diluent: The Acylation Diluent has a freezing point of 18.5°C. To ensure that the Acylation Diluent is liquid when being used, it must be ensured that the Acylation Diluent has reached room temperature and forms a homogeneous, crystal-free solution before being used. Alternative the Acylation Diluent can be stored at room temperature (20 – 25°C) separate from the other kit components.

Acylation Reagent: Reconstitute **each vial with 2 mL Acylation Diluent.**

The Acylation Reagent has to be prepared freshly prior to the assay (not longer than 1 hour in advance). If more than 1.25 mL is needed pool the contents of 2 or 3 vials and mix thoroughly. **Prepared solution can be stored for 1 month at 2-8 °C**

STORAGE AND STABILITY

Store the reagents at 2 - 8 °C until expiration date. Do not use components beyond the expiry date, indicated on the kit labels. Do not mix various lots of any kit component within an individual assay.

TEST PROCEDURE (ALLOW ALL REAGENTS TO REACH ROOM TEMP. BEFORE USE). Read the manual very carefully and get familiar with the supplied reagents, preparations and their use in this test. **Allow all reagents to reach room temperature and mix thoroughly by gentle inversion before use. Duplicate determinations are recommended.**

Preparation, and acylation

1. Pipette 25 µL of calibrators, 25 µL of controls, 25 µL of plasma unknowns, or 25 µL of culture supernatants or 10 µL of urine unknowns, or 50 µL of supernatant from the release test* into the respective wells of the Reaction Plate.
2. Add 25 µL of Acylation Buffer to all wells.
3. Add 25 µL of Acylation Reagent to all wells.
4. Incubate for 45 min at RT (20-25°C) on a shaker (approx. 600 rpm).
5. Add 200 µL of distilled water to all wells.
6. Incubate for 15 min. at RT (20-25°C) on a shaker (approx. 600 rpm).
7. Take 25 µL of the prepared calibrators, controls and unknowns for the Histamine ELISA

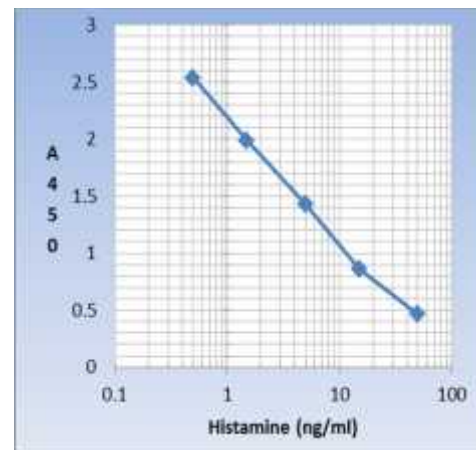
* For the release test the Histamine Release supplementary kit should be used.

Histamine ELISA PROCEDURE:

1. Pipette **25 µL** of **standards**, controls and samples into the respective pre-coated histamine Microtiter Strips.
2. Pipette **100 µL** of the **histamine Antiserum** into all wells.
3. Cover the plate with Adhesive Foil. **Incubate for 3 hours at RT (20-25°C)** on a shaker (approx. 600 rpm). **Alternatively: shake the Histamine Microtiter Strips** briefly by hand and incubate for **15-20 hrs** at 2 – 8 °C.
4. Remove the foil and discard or aspirate the contents of the wells and wash each well **4 times** thoroughly with **300 µL Wash Buffer**. Blot dry by tapping the inverted plate on absorbent material.
5. Pipette **100 µL** of **Enzyme Conjugate** into all wells.
6. Cover the plate with **Adhesive Foil** and incubate **30 min at RT (20-25°C)** on a **shaker** (approx. 600 rpm).
7. Remove the foil and discard or aspirate the contents of the wells and **wash each well 4 times** thoroughly with **300 µL Wash Buffer**. Blot dry by tapping the inverted plate on absorbent material.
8. Pipette **100 µL** of **Substrate** into all wells.
9. Incubate **20-30 min** at RT (20-25°C) on a shaker (approx. 600 rpm). **Avoid exposure to direct sun light!**
10. Pipette **100 µL** of Stop Solution into all wells.
11. Read the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to 450 nm and a reference wavelength between 620 nm and 650 nm.

Typical ELISA Results

	Average A450	OD/OD max	Results
Stds A (0 ng/ml)	2.560	100	
Stds B (0.5 ng/ml)	2.385	93	
Stds C (1.5 ng/ml)	1.988	78	
Stds D (5 ng/ml)	1.432	56	
Stds E (15 ng/ml)	0.862	34	
Stds F (50 ng/ml)	0.461	18	
Sample 1	1.651	64	3.3
Sample 2	0.932	36	13



3-ADI-ELISA

Calculation of results:

The calibration curves are obtained by plotting the absorbance readings (calculate the mean absorbance) of the standards (linear, y-axis) against the corresponding standard concentrations (logarithmic, x-axis). Use a non-linear regression for curve fitting (e.g. spline, 4-parameter). The concentrations of the undiluted plasma samples and the controls can be read directly from the standard curve.

	Concentration of the standards					
	A	B	C	D	E	F
Histamine (ng/ml= ug/L)	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= (nmol/L)					