

**Aviva Systems Biology**

5754 Pacific Center Blvd., Suite 201

San Diego, CA 92121, USA

Tel: 858-552-6979 Fax: 858-552-6975

Email: [info@avivasysbio.com](mailto:info@avivasysbio.com)

[www.avivasysbio.com](http://www.avivasysbio.com)



**Instructions for use**  
**Histamine ELISA Kit**  
**Catalog # OKDA00076**

**96 wells**

**For Research Use Only**



## Histamine ELISA

### 1. Introduction

#### 1.1 Intended use and principle of the test

Enzyme Immunoassay for the quantitative determination of Histamine in plasma and urine.

In combination with the supplementary kit *Histamine Release* (for details contact your local supplier), the assay can be used for the measurement of histamine release in heparinized whole blood.

In the first part of the procedure, 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 analyte concentrations in the standards, controls and samples and the solid phase bound analyte compete for a fixed number of antibody binding sites. After the system is in equilibrium, free antigen and free antigen-antibody complexes are removed by washing. The antibody bound to the solid phase is detected by an anti-goat 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 standard curve prepared with known standard concentrations.

#### 1.2 Clinical application

Histamine belongs to the biogenic amines and is synthesized by decarboxylation from the amino acid histidine. It is synthesized by mast cells, basophils, platelets, histaminergic neurons, and enterochromaffine cells, where it is stored intracellularly in vesicles and released on stimulation.

Histamine acts by binding to its 4 receptors (H1R, H2R, H3R and H4R) on target cells in various tissues. It causes smooth muscle cell contraction, vasodilatation, increased vascular permeability and mucus secretion, tachycardia, alterations of blood pressure, and arrhythmias.

In humans, histamine is one of the most important mediators and takes part in the initial phase of an anaphylactic reaction ("immediate type" allergy).

Of clinical interest is also the quantification of the histamine *release* from basophilic leucocytes in allergies.

Therapeutic consequences should never be based on laboratory results alone even if all test results are in agreement with the items as under point "Procedural cautions, guidelines and warnings". Any laboratory result is only a part of the total clinical picture of the patient.

Only in cases where the laboratory results are in an acceptable agreement with the overall clinical picture of the patient it can be used for therapeutic consequences.

The test result itself should never be the sole determinant for deriving any therapeutic consequences.

### 2. Procedural cautions, guidelines, warnings and limitations

#### 2.1 Precautions, guidelines and warnings

- (1) This kit is intended for professional use only. Users should have a thorough understanding of this protocol for the successful use of this kit. Only the test instruction provided with the kit is valid and has to be used to run the assay. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
- (2) This assay was validated for certain types of sample as indicated in *Intended Use* (please refer to Chapter 1). Any off-label use of this kit is in the responsibility of the user and the manufacturer cannot be held liable.
- (3) Reagents of this kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
- (4) The principles of Good Laboratory Practice (GLP) have to be followed.
- (5) In order to reduce exposure to potentially harmful substances, wear lab coats, disposable protective gloves and protective glasses where necessary.
- (6) All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
- (7) For dilution or reconstitution purposes, use deionized, distilled, or ultra-pure water.
- (8) The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch with desiccant and used in the frame provided.
- (9) Duplicate determination of sample is highly recommended to be able to identify potential pipetting errors.
- (10) Once the test has been started, all steps should be completed without interruption. Make sure that the required reagents, materials and devices are prepared ready at the appropriate time.
- (11) Incubation times do influence the results. All wells should be handled in the same order and time intervals.

- (12) To avoid cross-contamination of reagents, use new disposable pipette tips for dispensing each reagent, sample, standard and control.
- (13) A standard curve must be established for each run.
- (14) The controls should be included in each run and fall within established confidence limits. The confidence limits are listed in the QC-Report provided with the kit.
- (15) Do not mix kit components with different lot numbers within a test and do not use reagents beyond expiry date as shown on the kit labels.
- (16) Avoid contact with Stop Solution containing 0.25 M H<sub>2</sub>SO<sub>4</sub>. It may cause skin irritation and burns. In case of contact with eyes or skin, rinse off immediately with water.
- (17) Some reagents contain sodium azide (NaN<sub>3</sub>) as preservative. In case of contact with eyes or skin, rinse off immediately with water. NaN<sub>3</sub> may react with lead and copper plumbing to form explosive metal azides. When disposing reagents, flush with a large volume of water to avoid azide build-up.
- (18) TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them.
- (19) For information on hazardous substances included in the kit please refer to Material Safety Data Sheet (MSDS). The Material Safety Data Sheet for this product is made available directly on the website of the manufacturer or upon request.
- (20) The expected reference values reported in this test instruction are only indicative. It is recommended that each laboratory establishes its own reference intervals.
- (21) The results obtained with this test kit should not be taken as the sole reason for any therapeutic consequence but have to be correlated to other diagnostic tests and clinical observations.
- (22) Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.

## 2.2 Limitations

Any inappropriate handling of samples or modification of this test might influence the results.

### 2.2.1 Interfering substances

#### Plasma

Samples containing precipitates or fibrin strands or which are haemolytic or lipemic might cause inaccurate results.

#### 24-hour urine

Please note the sample preparation! If the percentage of the final concentration of acid is too high, the buffer capacity of the Acylation Buffer is insufficient. As a consequence histamine will not be acylated quantitatively.

### 2.2.2 Drug interferences

There are no known substances (drugs) which ingestion interferes with the measurement of histamine level in the sample.

### 2.2.3 High-Dose-Hook effect

No hook effect was observed in this test.

## 3. Storage and stability

Store the unopened reagents at 2 - 8 °C until expiration date. Do not use components beyond the expiry date indicated on the kit labels. Once opened the reagents are stable for 1 month when stored at 2 - 8 °C. Once the resealable pouch has been opened, care should be taken to close it tightly with desiccant again.

## 4. Materials

### 4.1 Contents of the kit

<b>BA D-0090</b>	<b>FOILS</b>	<b>Adhesive Foil</b> - Ready to use
Contents:	Adhesive Foils in a resealable pouch	
Volume:	1 x 4 foils	
<b>BA E-0030</b>	<b>WASH-CONC 50x</b>	<b>Wash Buffer Concentrate</b> - Concentrated 50x
Contents:	Buffer with a non-ionic detergent and physiological pH	
Volume:	1 x 20 ml/vial, light purple cap	
<b>BA D-0024</b>	<b>REAC-PLATE</b>	<b>Reaction Plate</b> - Ready to use
Contents:	1 x 96 well plate, empty in a resealable pouch	

<b>BA E-1040</b>	<b>CONJUGATE</b>	<b>Enzyme Conjugate</b> - Ready to use
Contents:	Rabbit anti-goat immunoglobulins conjugated with peroxidase	
Volume:	1 x 12 ml/vial, red cap	
<b>BA E-0055</b>	<b>SUBSTRATE</b>	<b>Substrate</b> - Ready to use
Contents:	Chromogenic substrate containing tetramethylbenzidine, substrate buffer and hydrogen peroxide	
Volume:	1 x 12 ml/black vial, black cap	
<b>BA E-0080</b>	<b>STOP-SOLN</b>	<b>Stop Solution</b> - Ready to use
Contents:	0.25 M sulfuric acid	
Volume:	1 x 12 ml/vial, light grey cap	
<b>BA E-1031</b>	<b>HIS</b>	<b>Histamine Microtiter Strips</b> - Ready to use
Contents:	1 x 96 well (12x8) antigen precoated microwell plate in a resealable pouch with desiccant.	
<b>BA E-1010</b>	<b>HIS-AS</b>	<b>Histamine Antiserum</b> - Ready to use
Contents:	Goat Anti- Histamine antibody, blue coloured	
Volume:	1 x 12 ml/vial, blue cap	

**Standards and Controls** - Ready to use

Cat. no.	Component	Colour /Cap	Concentration ng/ml	Concentration nmol/l	Volume/ Vial
<b>BA E-1001</b>	<b>STANDARD A</b>	white	0	0	4 ml
<b>BA E-1002</b>	<b>STANDARD B</b>	light yellow	0.5	4.5	4 ml
<b>BA E-1003</b>	<b>STANDARD C</b>	orange	1.5	13.5	4 ml
<b>BA E-1004</b>	<b>STANDARD D</b>	dark blue	5	45	4 ml
<b>BA E-1005</b>	<b>STANDARD E</b>	light grey	15	135	4 ml
<b>BA E-1006</b>	<b>STANDARD F</b>	black	50	450	4 ml
<b>BA E-1051</b>	<b>CONTROL 1</b>	light green	Refer to vial for expected value and acceptable range!		4 ml
<b>BA E-1052</b>	<b>CONTROL 2</b>	dark red			4 ml

Conversion: Histamine (ng/ml) x 9 = Histamine (nmol/l)

Contents: Acidic buffer spiked with defined quantity of Histamine

<b>BA E-1011</b>	<b>ACYL-BUFF</b>	<b>Acylation Buffer</b> - Ready to use
Contents:	TRIS-buffer containing a non-mercury preservative	
Volume:	1 x 4 ml/vial, pink cap	
<b>BA E-1012</b>	<b>ACYL-REAG</b>	<b>Acylation Reagent</b> - Lyophilized
Contents:	Lyophilized acylation reagent	
Volume:	4 vials, purple cap	
<b>BA E-0085</b>	<b>ACYL-SOLV</b>	<b>Acylation Solvent</b> - Ready to use
Contents:	Ethanol	
Volume:	1 x 10 ml/vial, brown cap	

Hazards  
identification:



H225 Highly flammable liquid and vapour.

#### 4.2 Additional materials and equipment required but not provided in the kit

- Calibrated precision pipettes to dispense volumes between 10 - 300 µl; 1.25 ml
- Microtiter plate washing device (manual, semi-automated or automated)
- ELISA reader capable of reading absorbance at 450 nm and if possible 620 - 650 nm
- Microtiter plate shaker (shaking amplitude 3 mm; approx. 600 rpm)
- Absorbent material (paper towel)
- Water (deionized, distilled, or ultra-pure)
- Vortex mixer

## 5. **Sample collection and storage**

In general the repeated freezing and thawing of samples should be avoided.

### **Plasma**

Whole blood should be collected by venipuncture into centrifuge tubes containing EDTA as anti-coagulant (e.g. Monovette™ or Vacuette™ for plasma) and centrifuged at room temperature immediately after collection.

Haemolytic and especially lipemic samples should not be used for the assay.

Storage: up to 6 hours at 2 - 8 °C, for longer period (up to 6 month) at -20 °C.

### **Urine**

Spontaneous urine or 24-hour urine, collected in a bottle containing 10 - 15 ml of 6 M HCl, can be used.

*If 24-hour urine is used please record the total volume of the collected urine.* If the percentage of the final concentration of acid is too high, the buffer capacity of the Diluent is insufficient. As a consequence interfering factors are not extracted quantitatively.

Storage: up to 6 hours at 2 - 8 °C, for longer periods (up to 6 month) at -20 °C.

Avoid exposure to direct sunlight.

### **Whole Blood**

The release of histamine is performed with heparinized whole blood. For further information please refer to the instructions for use of the add-on kit **Histamine Release** (for details contact your local supplier).

## 6. **Test procedure**

Allow all reagents and samples to reach room temperature and mix thoroughly by gentle inversion before use. Duplicate determinations are recommended.

The binding of the antibody and the enzyme conjugates and the activity of the enzyme used are temperature dependent, and the absorbance values may vary if a thermostat is not used. The higher the temperature, the higher the absorption values will be. Varying incubation times have similar influences on the absorbance. 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*

### 6.1 **Preparation of reagents**

#### **Wash Buffer**

Dilute the 20 ml Wash Buffer Concentrate with water (deionized, distilled or ultra-pure) to a final volume of 1000 ml.

Storage: 1 month at 2 - 8 °C

#### **Acylation Solution**

The Acylation Solution has to be prepared freshly prior to the assay:

Reconstitute each vial of the Acylation Reagent (BA E-1012) with 2 ml Acylation Solvent (BA E-0085). Please make sure that it is completely dissolved before use.

If more than 2 ml are needed, pool the contents of the individual vials and mix thoroughly.

Discard after use!

### 6.2 **Sample preparation and acylation**

1.	Pipette <b>25 µl</b> of <b>standards, controls</b> and <b>plasma samples</b> , <b>10 µl</b> of <b>urine samples</b> , or <b>50 µl</b> of <b>supernatant</b> from the <b>release test*</b> into the respective wells of the <b>Reaction Plate</b> .
2.	Add <b>25 µl</b> of <b>Acylation Buffer</b> to all wells.
3.	Add <b>25 µl</b> of <b>Acylation Solution</b> (refer to 6.1) to all wells.
4.	Incubate for <b>45 min</b> at <b>RT</b> (20 - 25 °C) on a <b>shaker</b> (approx. 600 rpm).
5.	Add <b>100 µl</b> of <b>water</b> (deionized, distilled, or ultra-pure) to all wells.
6.	Incubate for <b>15 min</b> at <b>RT</b> (20 - 25 °C) on a <b>shaker</b> (approx. 600 rpm).
	Take <b>25 µl</b> of the prepared <b>standards, controls and samples</b> for the <b>Histamine ELISA</b>

\* For the **release test** the **Histamine Release** supplementary kit (for details contact your local supplier) has to be used.

### 6.3 Histamine ELISA

1.	Pipette <b>25 µl</b> of the <b>acylated standards, controls</b> and <b>samples</b> into the appropriate wells of the <b>Histamine Microtiter Strips</b> .
2.	Pipette <b>100 µl</b> of the <b>Histamine Antiserum</b> into all wells and cover plate with <b>Adhesive Foil</b> .
3.	Incubate for <b>3 h</b> at <b>RT</b> (20 - 25 °C) on a <b>shaker</b> (approx. 600 rpm). <b>Alternatively:</b> shake the <b>Histamine Microtiter Strips</b> briefly by hand and incubate for <b>20 - 25 h</b> at <b>2 - 8 °C</b> .
4.	Remove the foil. Discard or aspirate the contents of the wells. Wash the plate <b>4 x</b> by adding <b>300 µl</b> of <b>Wash Buffer</b> , <b>discarding</b> the content and <b>blotting dry each time</b> by tapping the inverted plate on absorbent material.
5.	Pipette <b>100 µl</b> of the <b>Enzyme Conjugate</b> into all wells.
6.	Incubate for <b>30 min</b> at <b>RT</b> (20 - 25 °C) on a <b>shaker</b> (approx. 600 rpm).
7.	Discard or aspirate the contents of the wells. Wash the plate <b>4 x</b> by adding <b>300 µl</b> of <b>Wash Buffer</b> , <b>discarding</b> the content and <b>blotting dry each time</b> by tapping the inverted plate on absorbent material.
8.	Pipette <b>100 µl</b> of the <b>Substrate</b> into all wells and incubate for <b>20 - 30 min</b> at <b>RT</b> (20 - 25 °C) on a <b>shaker</b> (approx. 600 rpm). <b>Avoid exposure to direct sunlight!</b>
9.	Add <b>100 µl</b> of the <b>Stop Solution</b> to each well and shake the microtiter plate to ensure a homogeneous distribution of the solution.
10.	<b>Read</b> the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to <b>450 nm</b> (if available a reference wavelength between 620 nm and 650 nm is recommended).

### 7. Calculation of results

Measuring range		Histamine
	Plasma	0.12 - 50 ng/ml
	Urine	0.3 - 125 ng/ml

The standard curve is 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, akima).

#### Plasma samples and controls:

The concentrations of the **plasma samples** and the **controls** can be read directly from the standard curve.

#### Urine samples:

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:

$$\mu\text{g}/24\text{h} = \mu\text{g}/\text{l} \times \text{l}/24\text{h}$$

#### Conversion

$$\text{Histamine (ng/ml)} \times 9 = \text{Histamine (nmol/l)}$$

#### Expected reference values

It is strongly recommended that each laboratory should determine its own reference values.

In a study conducted with apparently normal healthy adults, using the Histamine ELISA the following values are observed:

Plasma	24 hour-urine	Spontaneous urine
< 1 ng/ml	< 45 µg/d	< 45 µg/g creatinine

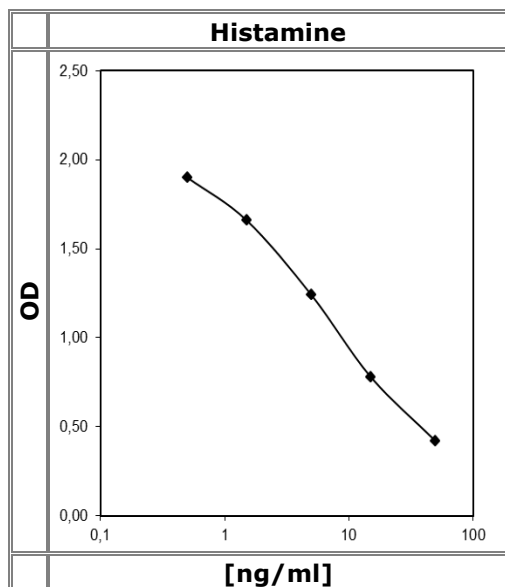
### 7.1 Quality control

It is recommended to use control samples according to national regulations. Use controls at both normal and pathological levels. The kit, or other commercially available, controls should fall within established confidence limits. The confidence limits of the kit controls are indicated in the QC-Report.

## 7.2 Typical standard curve



Example, do not use for calculation!



## 8. Assay characteristics

Analytical Sensitivity (Limit of Detection)	Histamine	
	Sensitivity Plasma	0.12 ng/ml
	Sensitivity Urine	0.30 ng/ml

Analytical Specificity (Cross Reactivity)	Substance	Cross Reactivity (%)
		Histamine
	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

Precision							
Intra-Assay	Sample	Range (ng/ml)	CV (%)	Inter-Assay	Sample	Range (ng/ml)	CV (%)
Histamine	1	9.7 ± 1.5	15	Histamine	1	9.9 ± 1.7	11.8
	2	18.6 ± 2.4	12.8		2	42.3 ± 3.4	7.9
Urine	1	1.2 ± 0.2	15.8	Control samples			
	2	5.0 ± 0.6	11.8				
Histamine	1	1.2 ± 0.2	15.8				
Plasma	2	5.0 ± 0.6	11.8				

Linearity		Range	Serial dilution up to	Range (%)
	Urine	4.33 - 70 ng/ml	1:16	90 - 124
	Plasma	0.74 - 8.48 ng/ml	1:16	85 - 106

Recovery		Range ng/ml	Mean (%)	Range (%)
	Urine	6.9 - 25.9 ng/ml	115	113 - 117
	Plasma	0.36 - 6.5 ng/ml	84	78 - 89

Method comparison versus ELISA	Urine	ELISA = 0.9 ELISA (LDN) - 3.1	r = 0.98; n = 29
	Plasma	ELISA = 1.0 ELISA (LDN) - 0.4	r = 0.99; n = 47













## 9. References/Literature

- (1) Yagci et al. TCTP/HRF pathway and angiogenesis: A feasible intercourse in chronic lymphocytic leukaemia. Leukemia Research, 37:665-670 (2013)
- (2) Coulson et al. Paracetamol (acetaminophen) attenuates in vitro mast cell and peripheral blood mononucleocyte cell histamine release induced by N-acetylcysteine. Clinical Toxicology, 48(2):111-114 (2010)
- (3) Rovere et al. Histamine and Selenium in Lung Cancer. Anticancer Research, 26: 2937-2942 (2006)

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 **For updated literature or any other information please contact your local supplier.**

### **Symbols:**

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Expiry date		Batch code		For in-vitro diagnostic use only!
	Consult instructions for use		Content		CE labelled
	Caution		Catalogue number		For research use only!