BioVendor Group

ENG

Instructions for Use:
# HISTAMINE ELISA

Catalogue number:
**RA19008R**

**For research use only!**

Example Version

BioVendor R&D

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CONTENT



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## HISTORY OF CHANGES

| Previous version | Current version |
|---|---|
| ENG.002.A | ENG.003.A |
| Chapter 2: This allows for the construction of one standard curve in duplicate and the assay of 33 samples in duplicate. | Chapter 2: This allows for the construction of one standard curve in duplicate and the assay of 36 samples in duplicate. |
| Modifying the format and structure of the document. | |
| Chapter 3: information on protective equipment added. | |
| Chapter 4 added. | |
| Chapter 5 changed. | |
| Items in chapter 6 added. | |
| Chapter 7 changed, chapter 7.3. new text added. | |
| Chapter 8 changed. | |
| Chapter 9 changed, chapter 9.5 Plate set-up changed. | |
| Chapter 11: change of values. | |
| Chapter 12: values and graphs changed. | |
| Chapter 14: new text added. | |
| Chapter 14: new text added. | |
| Chapter 17. Assay protocol changed. | |

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# 1. HISTAMINE ELISA

96 wells
Storage: -20°C
Expiry date: stated on the package

# 2. REAGENTS SUPPLIED

| REAGENTS | Quantity | Form |
|---|---|---|
| Antibody Coated Microtiter Strips (with mouse anti-Histamine monoclonal antibody, ready to use after thawing) | 1 | |
| Conjugate Solution (Histamine tracer) | 1 | lyophilized |
| Histamine Standard | 2 | liquid |
| Derivatization reagent | 2 | powder |
| Derivatization buffer | 1 | liquid |
| Quality Control sample | 2 | liquid |
| Dilution Buffer (EIA buffer) | 1 | lyophilized |
| Wash Buffer | 1 | liquid |
| Substrate Solution (Ellman´s reagent) | 2 | lyophilized |
| Tween 20 | 1 | liquid |
| Cover Sheet | 1 | |
| Template Sheet | 1 | |

Each kit contains sufficient reagents for 96 wells. This allows for the construction of one standard curve in duplicate and the assay of 36 samples in duplicate.

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# 3. PRECAUTION FOR USE

Users are recommended to read all instructions for use before starting work.

Each time a new pipette tip is used, aspirate a sample or reagent and expel it back into the same vessel. Repeat this operation two or three times before distribution in order to equilibrate the pipette tip.

- For research laboratory use only.
- Not for diagnostic use.
- Do not pipet liquids by mouth.
- Do not use kit components beyond the expiration date.
- Do not eat, drink or smoke in area in which kit reagents are handled.
- Avoid splashing.

The total amount of reagents contains less than 100 µg of sodium azide. Flush the drains thoroughly to prevent the production of explosive metal azides.

Wearing lab gloves, laboratory coat and eye protection glasses is recommended when assaying kit materials and samples.

**Temperature**
Unless otherwise specified, all the experiments are done at room temperature (RT), that is around +20°C. Working at +25°C or more affects the assay and decreases its efficiency

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# 4. BACKGROUND

## 4.1 Acetylcholinesterase AChE® Technology

Acetylcholinesterase (AChE®), the enzymatic label for ELISA, has the fastest turnover rate of any enzymatic label. This specific AChE® is extracted from the electric organ of the electric eel, Electrophorus electricus, and it is capable of providing a rapid catalytic turnover during the generation of the electrochemical discharges. The use of AChE® as enzymatic label for ELISA is patented by the French academic research Institute CEA [1, 2, 3].
AChE® assays are revealed with Ellman's reagent, which contains acetylthiocholine as a substrate. The final product of the enzymatic reaction (5-thio-2-nitrobenzoic acid), is bright yellow in color and can be read at 405-414 nm using a spectrophotometer. AChE® offers several advantages over other commonly used enzymes used in ELISAs:

### 4.1.1 Kinetic superiority and high sensitivity:

AChE® shows true first-order kinetics with a turnover of 64,000 sec-1. That is nearly 3 times faster than Horse Radish Peroxidase (HRP) or alkaline phosphatase. AChE® provides greater sensitivity than other labelling enzymes.

### 4.1.2 Low background

Non-enzymatic hydrolysis of acetylthiocholine in buffer is essentially absent. Thus, AChE® ensures a very low background and an increased signal/noise ratio compared to other substrate of enzymes that are inherently unstable.

### 4.1.3 Wide dynamic range

AChE® is a stable enzyme and its activity remains constant for many hours, unlike other enzymes, its substrate is not suicidal. This permits simultaneous assays of high diluted and very concentrated samples.

### 4.1.4 Versatility:

AChE® is a completely stable enzyme, unlike peroxidase which is suicidal. Thus, if a plate is accidentally dropped after dispatch of the AChE® substrate (Ellman's reagent), one only needs to wash the plate, add fresh Ellman's reagent and proceed with a new development. Otherwise, the plate can be stored at +4°C while waiting for technical advice from the Technical support.

### 4.2 Histamine

Histamine is a natural amine present in many animal and human tissues. It was considered to be the mediator of immediate allergy.

Histamine plays a role in various physiological processes, such as control of gastric acid secretion, neurotransmission and modulation of inflammatory and immunological reactions

## 5. PRINCIPLE OF THE ASSAY

The enzymatic immunoassay (ELISA) is based on the competition between unlabelled (free) Histamine (standards / QC / samples) and acetylcholinesterase (AChE) linked to Histamine (Tracer) for limited specific mouse anti-Histamine antibody sites.

As a former step of this assay, Histamine is derivatized to increase the affinity of Histamine to the antibody and consequently increase the sensitivity of the assay.

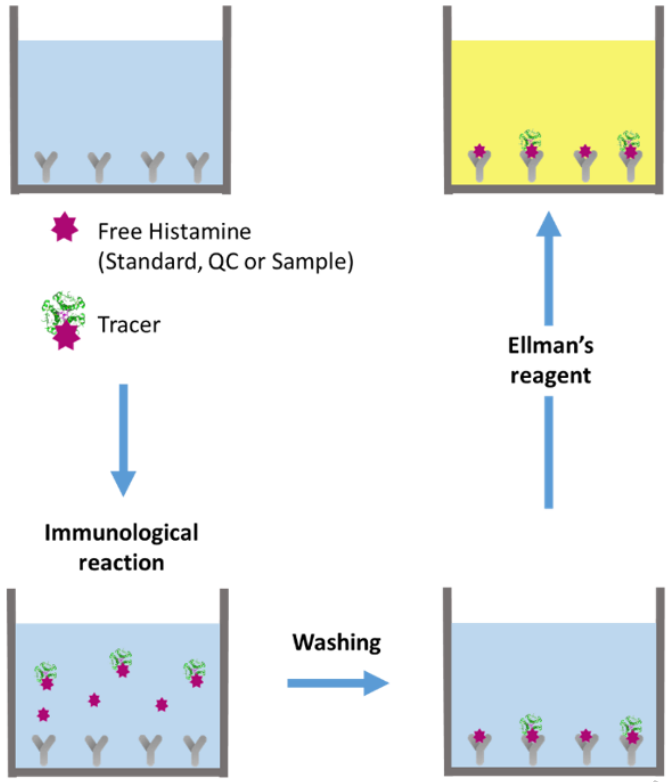
Tracer and free Histamine are incubated in wells which have been precoated with a mouse anti-Histamine antibody. The plate is washed to remove any unbound reagent, then Ellman's Reagent (enzymatic substrate for AChE and chromogen) is added to the wells.

The AChE tracer acts on the Ellman's Reagent to form a yellow compound that strongly absorbs at 414 nm.

The intensity of the color, determined by spectrophotometry, is proportional to the amount of tracer bound to the well and is inversely proportional to the amount of free Histamine present in the well during the immunological incubation:

The principle of the assay is summarised below:

Example Version

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## 6. MATERIAL REQUIRED BUT NOT PROVIDED

In addition to standard laboratory equipment, the following material is required:

- Anhydrous N,N-Dimethylformamide (DMF)(*) (if not available, DMS0)
- Precision micropipettes (20 to 1000 µl)
- Multichannel pipette and disposable tips 30-300 µl
- Spectrophotometer plate reader (405 or 414 nm filter)
- Microplate washer (or wash bottles)
- UltraPure water
- Orbital microplate shaker
- Polypropylene tubes (**no glass tubes**)
- For plasma sample: EDTA tubes
- For solid and liquid biological samples: $HClO_4$ & NaOH

(*) Make sure that the bottle of DMF has been opened for a short period of time. This point is important to get a good derivatization rate

Water used to prepare all ELISA reagents and buffers must be UltraPure (deionized & free from organic contaminant traces).
Otherwise, organic contamination can significantly affect the enzymatic activity of the tracer Acetylcholinesterase (AChE).
Do not use distilled water, HPLC-grade water or sterile water.

# 7. SAMPLE COLLECTION AND PREPARATION

## 7.1 General precautions

All samples must be free from organic solvents prior to assay.
Samples should be assayed immediately after collection or should be stored at -20°C.

This assay may be used to measure Histamine in samples such as plasma, urine, culture supernatants as well as liquid (e.g. broncho-alveolar lavage fluids) or solid (brain, nervous tissues) biological samples after extraction. Please refer to the appropriate paragraph for your sample preparation protocol.

## 7.2 Plasma

Collect blood samples in tubes containing EDTA. Centrifuge the samples at 1,600 g for 20 minutes. Collect plasma and keep at -20°C until assay. Thaw the sample on the day of the assay, vortex and centrifuge it at 1,600 g for 20 minutes to eliminate the fibrin.

No prior extraction procedure is necessary to measure Histamine in plasma samples. If necessary, plasma samples may be diluted in Histamine EIA buffer before derivatization (see below).

## 7.3 Urine or culture supernatants

Collect samples in polypropylene tubes. Store the samples at -20°C until assay. No prior extraction is necessary to measure Histamine in such samples.

## 7.4 Liquid or solid biological samples

### 7.4.1 Solid samples

For **solid** samples, we recommend addition of $HClO_4$ which will precipitate large proteins, while Histamine will remain in solution. Samples must be extracted at room temperature with 0.1M Perchloric Acid final concentration (10 µl/mg of tissue).
Homogenize and then centrifuge at 10,000 g for 5 minutes. Collect the supernatant.

### 7.4.2 Liquid samples

**Liquid** samples can be stored at -20°C just after collection in polypropylene vials. Before assaying, you have to filter liquid samples through a 0.22 µm filter in a tube containing $HClO_4$ 1M to get a final $HClO_4$ concentration of 0.1 M (for example: your tube must contain 20 µl $HClO_4$ 1M for 200 µl of sample).
Homogenize and then centrifuge at 10,000 g for 5 minutes. Collect the supernatant

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# 8. REAGENT PREPARATION

Each kit contains sufficient reagents for 96 wells. This allows for the construction of one standard curve in duplicate and the assay of 36 samples in duplicate.

If you want to use the kit in two times, we provide one additional vial of Standard, one of Derivatization Reagent, one of Quality Control and one of Ellman's Reagent.

All reagents need to be brought to room temperature (around+20°C) prior the use in assay.

## 8.1 Dilution Buffer (Histamine ELISA Buffer)

Reconstitute the Histamine ELISA Buffer with 25 ml of UltraPure water. Allow buffer to stand for 5 minutes or until it is completely dissolved. Mix buffer thoroughly by gentle inversions.
Stability at 4°C: 1 week

## 8.2 Histamine Standard

The Assay Buffer used to prepare the standards depends on the sample to be assayed:

- for plasma or urine samples, prepare the Standards using the Histamine ELISA Buffer,
- for culture supernatant samples, prepare the Standards using the same culture medium as for the sample,
- for extracted liquid or solid samples, prepare the Standards in 0.1 M $HClO_4$.

Prepare one Histamine Standard vial with 900 µl of Assay Buffer. Mix carefully 3 times with the pipet tip.

The obtained solution, called S0, has a concentration of 500 nM.

Take 8 polypropylene tubes (for the eight standards S1 to S8) and prepare the standards by serial dilutions as follow:

| Standard | Volume of Standard | Volume of Assay Buffer | Standard concentration |
|---|---|---|---|
| S1 | 100 µL of S0 | 900 µL | 50 nM |
| S2 | 500 µL of S1 | 500 µL | 25 nM |
| S3 | 500 µL of S2 | 500 µL | 12.5 nM |
| S4 | 500 µL of S3 | 500 µL | 6.25 nM |
| S5 | 500 µL of S4 | 500 µL | 3.13 nM |
| S6 | 500 µL of S5 | 500 µL | 1.56 nM |
| S7 | 500 µL of S6 | 500 µL | 0.78 nM |
| S8 | 500 µL of S7 | 500 µL | 0.39 nM |

Stability at 4°C: 24 hours

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### 8.3 Histamine Quality Control

Prepare one histamine Quality Control vial with 900 µL of assay medium, as for the standard. Then dilute 100 µL of QC in 900 µL of assay medium. The final concentration of this QC is labelled on the vial.

Stability at 4°C: 24 hours

### 8.4 Derivatization reagent

Before use, reconstitute the vial with 1 ml of N- N-dimethylformamide (DMF). The kit was validated with fresh DMF; if not available, one can use 1 ml DMSO. Vortex the content, and place the vial in a water bath at 37 °C. The resuspended content can contain tiny particles. This will not interfere with the assay. This reagent cannot be stored.

### 8.5 Histamine-AChE Tracer

Reconstitute the vial with 10 ml of UltraPure water. Allow it to stand 5 minutes until completely dissolved and then mix thoroughly by gentle inversion.

Stability at +4°C: 1 month

### 8.6 Wash Buffer

Dilute 2 ml of concentrated Wash Buffer with 800 ml of UltraPure water. Add 400 µl of Tween 20. Use a magnetic stirring bar to mix the contents.

Stability a +4°C: 1 month

### 8.7 Substrate Solution (Ellman's Reagent)

5 minutes before use (development of the plate), reconstitute one vial of Ellman's Reagent with 50 ml of UltraPure water. The tube contents should be thoroughly mixed.

Stability at 4°C and in the dark: 24 hours

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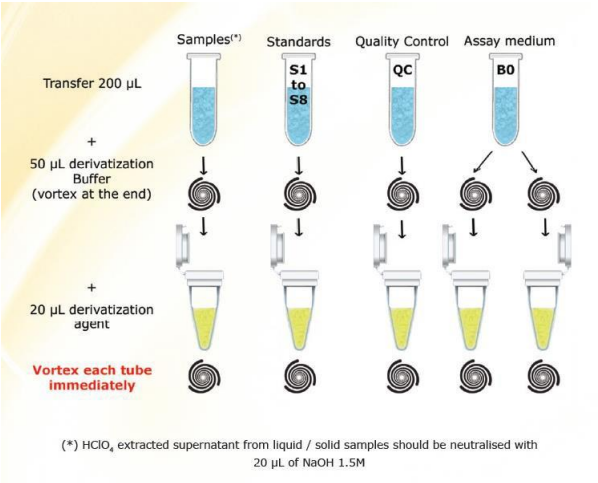
# 9. ASSAY PROCEDURE

It is recommended to perform the assay in duplicate and to follow the instructions hereafter.
Before derivatization, highly concentrated samples may be diluted in Histamine ELISA buffer (plasma, urine) or in assay medium (cell culture medium, $HClO_4$, …)
The assay procedure depends of the sample to be assayed

## 9.1 Sample derivatization

Illustrated derivatization protocol – please follow carefully the detailed protocol hereafter.




## 9.2 Histamine EIA buffer, plasma, urine, culture supernatant

- Prepare one polypropylene tube for each standard, quality control and samples. In these polypropylene tubes, distribute:
  - 200 µl of standard, quality control or sample,
  - 50 µl of derivatization buffer
- In two polypropylene tubes that will allow evaluation of maximum binding (B0), distribute:
  - 200 µl of assay medium
  - 50 µl of derivatization buffer
- Vortex all tubes
- Then tube by tube:
  - add 20 µl of derivatization reagent
  - vortex each tube immediately.

## 9.3 Liquid or solid biological sample:

- Prepare one polypropylene tube for each standard, quality control and samples. In these polypropylene tubes, distribute:
  - 200 µl of standard, quality control or sample,
  - 20 µl of 1.5M NaOH
  - 50 µl of derivatization buffer
- In two polypropylene tubes that will allow evaluation of maximum binding (B0), distribute:
  - 200 µl of assay medium
  - 20 µl of 1.5M NaOH
  - 50 µl of derivatization buffer
- Vortex all tubes
- Then tube by tube:

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- add 20 µl of derivatization reagent
- vortex each tube immediately.

### 9.4 Plate preparation

Prepare the Wash Buffer as indicated in the reagent preparation section.
Open the plate pouch and select enough strips for your assay and place the unused strips back in the pouch, store at +4°C for 1 month maximum.

Rinse each well 5 times with the Wash Buffer 300 µl/well.

Just before distributing the reagents and samples, remove the buffer from the wells by inverting the plate and blot the last drops by tapping it on paper towels.

### 9.5 Plate set-up

A plate set-up is suggested hereafter. The content of each well may be recorded on the template sheet provided at the end of this IFU.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | Bk | S7 | S3 | * | * | * | * | * | * | * | * | * |
| B | Bk | S6 | S2 | * | * | * | * | * | * | * | * | * |
| C | B0 | S6 | S2 | * | * | * | * | * | * | * | * | * |
| D | B0 | S5 | S1 | * | * | * | * | * | * | * | * | * |
| E | B0 | S5 | S1 | * | * | * | * | * | * | * | * | * |
| F | S8 | S4 | * | * | * | * | * | * | * | * | * | * |
| G | S8 | S4 | * | * | * | * | * | * | * | * | * | QC |
| H | S7 | S3 | * | * | * | * | * | * | * | * | * | QC |

Bk: Blank
S1-S8: Standards 1-8
QC: Quality Control

B0 : Maximum Binding
* : Samples

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### 9.6 Pipetting the reagents

Samples and reagents must reach room temperature prior performing the assay.

Use different tips for buffer, standard, sample, tracer and other reagents.

Before pipetting, equilibrate the pipette tips in each reagent. Do not touch the liquid already in the well when expelling with the pipette tip.

#### 9.6.1 Assay medium

Pool the 2 vials of B0 and dispense 100 µL to Maximum Binding (B0) wells.

#### 9.6.2 Histamine Standard

Dispense 100 µL of each of the eight derivatized standards S1 to S8 in duplicate to appropriate wells.

Start with the lowest concentration standard S8 and equilibrate the tip in the next higher standard before pipetting.

### 9.6.3 Histamine Quality Control and Samples

Dispense 100 µL in duplicate to appropriate wells.

### 9.6.4 Histamine AchE Tracer

Dispense 100 µl to each well, **except Blank (Bk) wells**.

### 9.7 Incubating the plate

Cover the plate with the cover sheet and incubate 24 hours at +4°C.

### 9.8 Developing and reading the plate

Reconstitute Ellman's reagent as mentioned in the Reagent preparation section.

Empty the plate by turning it over. Rinse each well 5 times with 300 µl Wash Buffer. At the end of the last washing step, empty the plate and blot the plate on a paper towel to discard any trace of liquid.

Add 200 µl of Ellman's reagent to each well. Cover the plate with an aluminium foil sheet and incubate in the dark at room temperature on an orbital shaker.

Gently wipe the bottom of the plate with a paper towel, and make sure that no liquid has splashed outside the wells.

Read the plate at a wavelength between 405 and 414 nm (yellow colour).
After addition of Ellman's reagent, the absorbance has to be checked periodically (every 30 minutes) until the maximum absorbance (B0 wells) has reached a minimum of
0.2 – 0.8 A.U. (blank subtracted).

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## 10. DATA ANALYSIS

Make sure that your plate reader has subtracted the absorbance readings of the blank wells (absorbance of Ellman's reagent alone) from the absorbance readings of the rest of the plate. If not, do it at this step.

- Calculate the average absorbance for B0, standard, quality control and sample wells.
- Calculate the B/B0 (%) for each standard, QC and sample (average absorbance of standards, QC or sample divided by average absorbance of B0) & multiply by 100.
- Using a semi-log graph paper, for each standard point, plot the B/B0 (%) on y axis versus the concentration on x axis. Draw a best-fit line through the points.
- To determine the concentration of your sample, the corresponding B/B0 (%) value has to fall within the linear range of the standard curve (usually comprised between 20% and 80%). Find the B/B0 (%) value on the y axis. Read the corresponding value on the x axis which is the concentration of your unknown sample.
- Samples which concentration determined on standard curve is greater than 50 nM should be re-assayed after appropriated dilution in Assay buffer prior to the derivatization step.

– Most plate readers are supplied with curve-fitting software capable of graphing these data (4-parameter or 5-parameter logistic fit). If you have this type of software, we recommend using it. Refer to it for further information.

Two vials of Quality Control are provided with this kit.
Your standard curve is validated only if the calculated concentration of the Quality Control obtained with the assay is +/- 25% of the expected concentration (see the Quality Control Sheet)

## 11. ACCEPTABLE RANGE

– B0 absorbance > 200 mA.U.
– IC50: 3,0 – 4,5 nM
– QC sample: ± 25% of the expected concentration (see the Quality Control Sheet)

## 12. TYPICAL RESULTS

The following data are for demonstration purpose only. Your data may be different and still correct. The data were obtained using all reagents as supplied in this kit under the following conditions: 1hour developing at +20°C, reading at 414 nm. A 4-parameter curve fitting was used to determine the concentrations.

| Standard or QC | Histamine ELISA Buffer | | Cell culture medium (RPMI) | | Perchloric acid (HClO4) | |
|---|---|---|---|---|---|---|
| | mA.U. | B/B0 (%) | mA.U. | B/B0 (%) | mA.U. | B/B0 (%) |
| B0 | 515 | 100,0 | 514 | 100,0 | 321 | 100,0 |
| 50 nM | 30 | 5,8 | 45 | 8,8 | 21 | 6,5 |
| 25 nM | 65 | 12,6 | 83 | 16,1 | 52 | 16,2 |
| 12.5 nM | 124 | 24,1 | 148 | 28,8 | 92 | 28,7 |
| 6.25 nM | 215 | 41,7 | 231 | 44,9 | 145 | 45,2 |
| 3.13 nM | 329 | 63,9 | 323 | 62,8 | 210 | 65,4 |
| 1.56 nM | 446 | 86,6 | 399 | 77,6 | 248 | 77,3 |
| 0.78 nM | 499 | 96,9 | 414 | 80,5 | 285 | 88,8 |
| 0.39 nM | 515 | 100,0 | 453 | 88,1 | 300 | 93,5 |
| QC 4 nM | 249 | 48,3 | | 48,4 | - | 77,6 |

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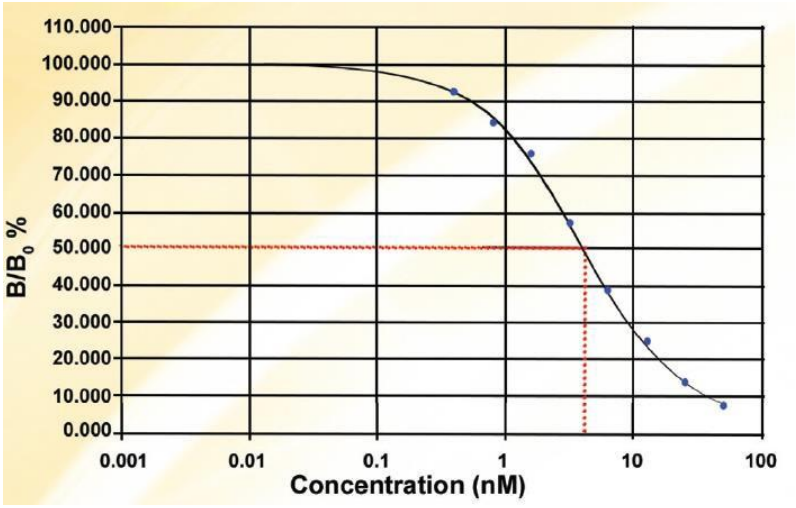
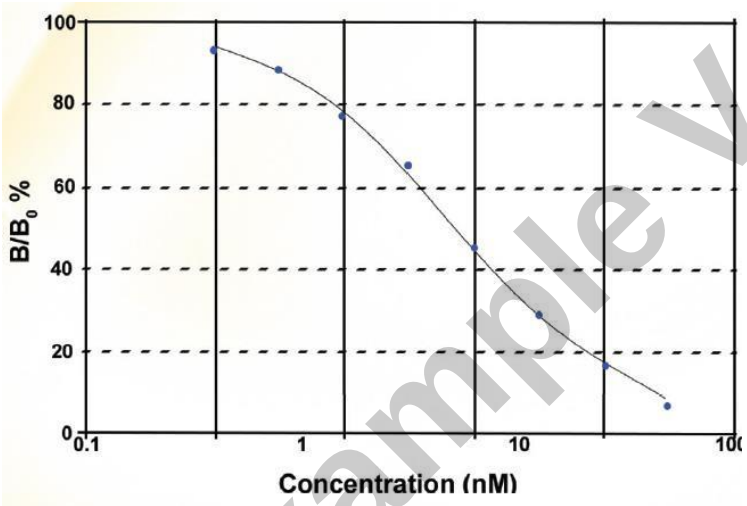
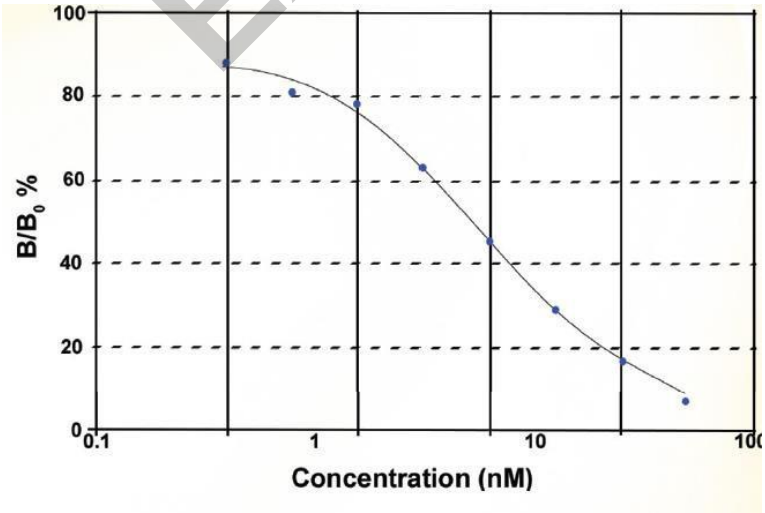



*Standard Curve in Histamine ELISA Buffer*




*Standard Curve in perchloric acid ($HClO_4$)*




*Standard Curve in cell culture medium (RPMI)*

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# 13. ASSAY CHARACTERISTICS

For additional information regarding the validation of immunoassay for protein biomarkers in biological samples, please refer to bibliography [4, 5].

## 13.1 Limit of detection (LOD)

0.5 nM (calculated as the concentration of Histamine corresponding to the B0 average minus three standard deviations)

## 13.2 Cross-reactivity

| Molecule/Species | Cross-reactivity |
|---|---|
| Histamine | 100 % |
| Histidine | < 0.01 % |
| 1-methylHistamine | 0.01 % |
| 3-methylHistamine | 0.038 % |
| Serotonin | < 0.01 % |

## 13.3 Intra & Inter-assay variation

| | Plasma QC (2.2 nM) | Plasma QC (8.6 nM) | Plasma QC (33.6 nM) |
|---|---|---|---|
| Mean value | - | 7.8 | 32.6 |
| Number of values | 30 | 30 | 30 |
| Intra-assay coeff. of variation (%) | 13.6 | 7.4 | 15.1 |
| Inter-assay coeff. of variation (%) | 19.4 | 8.0 | 15.8 |
| Recovery (%) | - | 90.7 | 97.1 |
| Confidence Interval | - | 90.71 ± 2.74 | 97.08 ± 5.78 |

| | Buffer QC (1 nM) | Buffer QC (5 nM) | Buffer QC (30 nM) |
|---|---|---|---|
| Mean value | 1.2 | 5.2 | 32.8 |
| Number of values | 30 | 30 | 30 |
| Intra-assay coeff. of variation (%) | 16.3 | 9.8 | 21.4 |
| Intra-assay coeff. of variation (%) | 28.9 | 11.6 | 21.4 |
| Recovery (%) | 123.2 | 103.4 | 109.5 |
| Confidence Interval | 123.15 ± 13.39 | 103.41 ± 4.51 | 109.47 ± 8.80 |

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| | Cell culture medium QC (5 nM) | $HClO_4$ QC (5 nM) |
|---|---|---|
| Mean value | 4.58 | 5.47 |
| Number of values | 20 | 20 |
| Intra-assay coeff. of variation (%) | 7.07 % | 6.43 % |
| Inter-assay coeff. of variation (%) | 5.20 % | 3.97 % |
| Recovery (%) | 91.60 % | 109.40 % |

### 13.4 Dilution test

| Day | Dilution | Histamine measured | Corrected concentrations | Recovery (%) | Mean |
|---|---|---|---|---|---|
| 1 | 1/1 | 30.79 | 30.79 | 94.39 | 136.41 |
| | 1/5 | 9.40 | 47.00 | 144.08 | |
| | 1/10 | 5.08 | 50.80 | 155.73 | |
| | 1/20 | 2.47 | 49.40 | 151.44 | |
| 2 | 1/1 | 32.15 | 32.15 | 98.56 | 113.65 |
| | 1/5 | 8.59 | 43.00 | 131.82 | |
| | 1/10 | 3.45 | 34.56 | 105.95 | |
| | 1/20 | 1.92 | 38.58 | 118.27 | |
| 3 | 1/1 | 31.94 | 31.94 | 97.92 | 130.08 |
| | 1/5 | 8.77 | 43.83 | 134.37 | |
| | 1/10 | 4.43 | 44.31 | 135.84 | |
| | 1/20 | 2.48 | 49.65 | 152.21 | |
| 4 | 1/1 | 26.56 | 26.56 | 81.42 | 115.24 |
| | 1/5 | 8.17 | 40.85 | 125.23 | |
| | 1/10 | 3.86 | 38.58 | 118.27 | |
| | 1/20 | 2.22 | 44.38 | 136.05 | |
| 5 | 1/1 | 22.15 | 22.15 | 67.90 | 107.72 |
| | 1/5 | 7.10 | 35.50 | 108.83 | |
| | 1/10 | 3.75 | 37.50 | 114.96 | |
| | 1/20 | 2.27 | 45.40 | 139.18 | |

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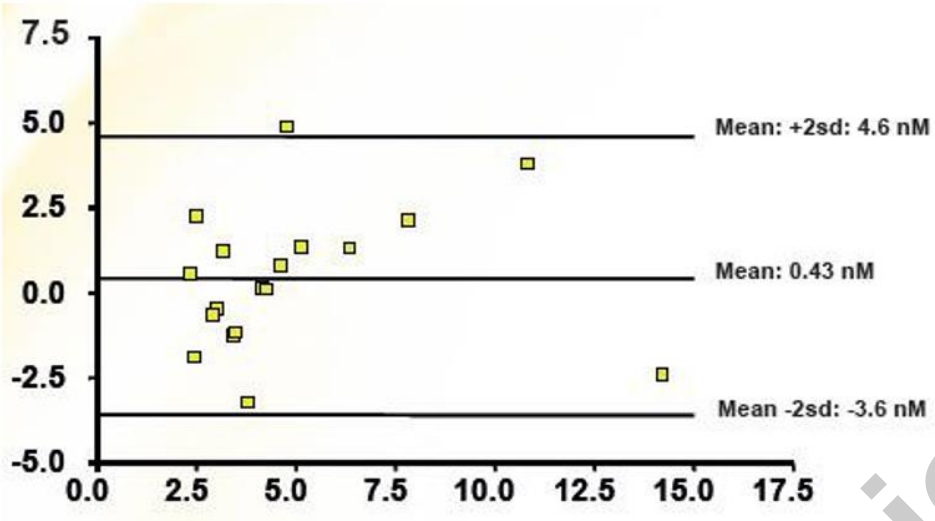
### 13.5 Recovery test

| | Histamine added (nM) | Histamine measured (nM) | Recovery (%) |
|---|---|---|---|
| Histamine ELISA Buffer | 5 | 5.2 | 103 |
| Cell culture medium | 5 | 5.04 | 101 |
| Cell culture medium & additives | 5 | 4.58 | 91.6 |
| $HClO_4$ | 5 | 5.47 | 109 |

## 13.6 Comparison with a reference method on 18 samples




## 13.7 Stability test (freezing / thawing)

| | Plasma QC1 | | Plasma QC2 | |
|---|---|---|---|---|
| | 32.6 nM | Recovery (%) | 7.8 nM | Recovery (%) |
| 1 cycle | 26.69 | 81.9 | 8.49 | 108.9 |
| 2 cycles | 29.42 | 90.3 | 9.63 | 123.5 |
| 3 cycles | 30.76 | 94.4 | 8.22 | 105.4 |
| 4 cycles | 24.26 | 74.4 | 6.73 | 86.3 |
| 5 cycles | - | - | 7.78 | 99.7 |
| Mean | 27.78 | - | 8.17 | - |
| Standard deviation | 2.90 | - | 1.06 | - |
| CV (%) | 10.42 | - | 12.93 | - |

| | Buffer QC1 | | Buffer QC2 | | Buffer QC3 | |
|---|---|---|---|---|---|---|
| | 30 nM | Recovery (%) | 5 nM | Recovery (%) | 1 nM | Recovery (%) |
| 1 cycle | 36.67 | 122.2 | 6.88 | 137.6 | 1.36 | 136.0 |
| 2 cycles | 29.63 | 98.8 | 4.17 | 83.4 | 1.29 | 129.0 |
| 3 cycles | 34.30 | 114.3 | 5.62 | 112.4 | 1.83 | 183.0 |
| 4 cycles | 29.83 | 99.4 | 5.69 | 113.8 | 1.31 | 131.0 |
| 5 cycles | 30.17 | 100.6 | 6.94 | 138.8 | 1.84 | 184.0 |
| Mean | 32.12 | - | 5.86 | - | 1.53 | - |
| Standard deviation | 3.19 | - | 1.13 | - | 0.28 | - |
| CV (%) | 9.93 | - | 19.6 | - | 18.56 | - |

Example Version

# 14. ASSAY TROUBLE SHOOTING

### 14.1 Absorbance values are too low:

- organic contamination of water
- one reagent has not been dispersed
- incorrect preparation / dilution
- assay performed before reagents reached room temperature
- reading time not long enough
- DMF used for derivatization was not fresh

### 14.2 High signal and background in all wells:

- inefficient washing
- overdeveloping (incubation time should be reduced)
- high ambient temperature

### 14.3 High dispersion of duplicates:

- poor pipetting technique
- irregular plate washing

### 14.4 $IC_{50}$ or QC concentrations not within expected range:

- wrong preparation of standards

### 14.5 Analysis of two dilutions of a biological sample are not coherent

- interfering substances are present. Sample must be purified prior to ELISA analysis (except plasma samples).

### 14.6 If a plate is accidentally dropped after dispatch of the AChE substrate (Ellman's Reagent) or if it needs to be revealed again:

- one only needs to wash the plate, add fresh Ellman's Reagent and proceed with a new development.
- otherwise, the plate can be stored at +4°C with Wash Buffer in wells while waiting for technical advice from info@biovendor.com.

These are a few examples of troubleshooting that may occur. If further information or explanation is needed, please contact info@biovendor.com

Example Version

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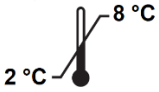
Example Version

## 16. EXPLANATION OF SYMBOLS

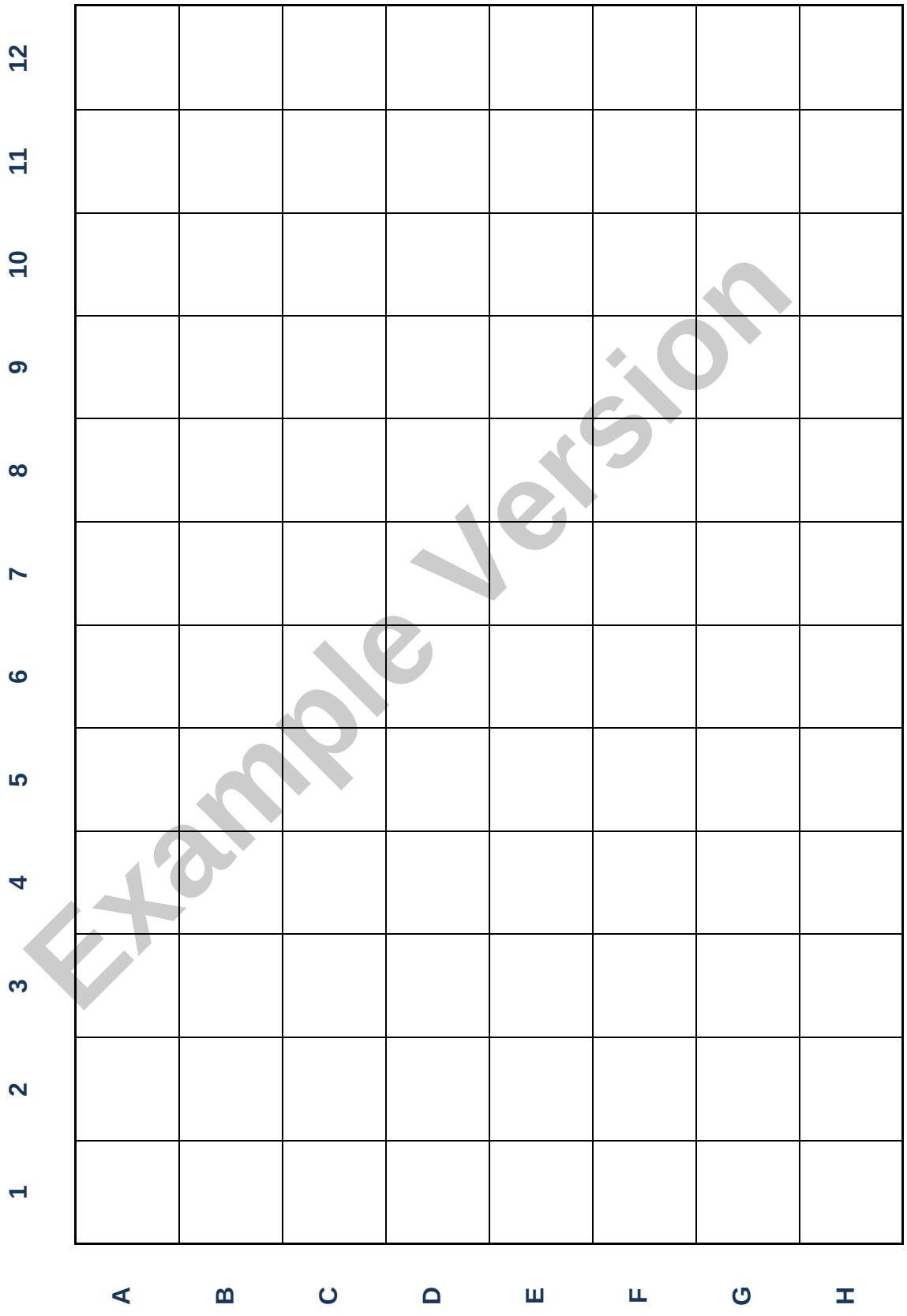
| | |
|---|---|
|   | Catalogue number |
|   | Batch code |
|  | Caution |
|  | Use by date |
|   | Temperature limit |
|  | Manufacturer |
|   | Read electronic instructions for use - eIFU |
|   | The content is sufficient for 96 tests |
|  | Biological risks |

Example Version

## 17. ASSAY PROCEDURE - SUMMARY

| Enzyme Immunoassay Protocol (volumes are in µL) | | | |
|---|---|---|---|
| Steps | Blank | Maximum binding | Standard, QC & sample |
| Derivatization | - | 200 µl of assay medium | 200 µl of standard, QC or sample |
| | - | 20 µl of 1.5M NaOH if assay medium is $HClO_4$ | |
| | - | 50 µl of derivatization agent | |
| | Vortex all tubes | | |
| | - | 20 µl of derivatization agent + immediate vortexing of each tube | |
| Wash the plate 5 times | | | |
| Distribution of reagents | - | 100 µl of derivatized solution | |
| | - | 100 µl of Tracer | |
| Cover plate, incubate at +4°C for 24 hours | | | |
| Wash strips 5 times & remove the liquid from the wells | | | |
| Developing | 200 µl of Ellman's Reagent | | |
| Incubate with an orbital shaker in the dark at room temperature | | | |
| Read the plate between 405 and 414 nm | | | |

Example Version




Example Version

Example Version

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