（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

**Catalog No：E-BC-K893-M**

**Specification：96T(40 samples)**

**Measuring instrument：Microplate reader(440-480 nm)**

**Detection range：2.44-200 μmol/L**

# **Elabscience®** Histamine Colorimetric Assay Kit

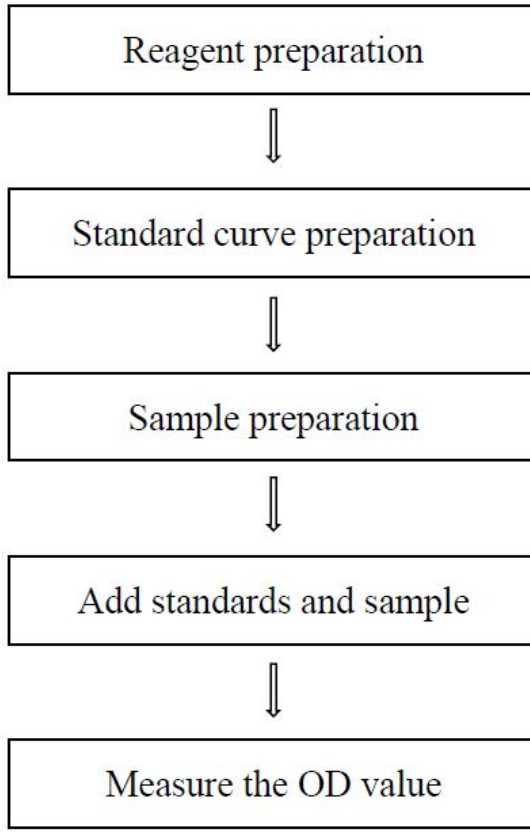
This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

# Table of contents

**Assay summary**



## Intended use

This kit can be used to measure histamine content in serum (plasma) tissue and cell samples.

## Detection principle

Histamine is an endogenous active substance that exists widely in nature and is an important mediator involved in inflammatory response and immune damage. The histamine content increases with the degree of spoilage of the organism. Histamine can produce chromogenic substances with chromogenic agents under the action of enzymes, which has a maximum absorption peak at 460 nm. The histamine content of the sample can be calculated by measuring the OD value at 460 nm.

## Kit components & storage

| Item | Component | Size (96 T) | Storage |
|---|---|---|---|
| Reagent 1 | Extraction Solution | 50 mL × 1 vial | -20℃, 12 months |
| Reagent 2 | Substrate | Powder × 5 vials | -20℃, 12 months, shading light |
| Reagent 3 | Buffer Solution | 14 mL × 1 vial | -20℃, 12 months, shading light |
| Reagent 4 | Chromogenic Solution | 1.5 mL × 2 vials | -20℃, 12 months, shading light |
| Reagent 5 | 1 mmol/L Standard Solution | 1.6 mL × 1 vial | -20℃, 12 months, shading light |
| | Microplate | 96 wells | No requirement |
| | Plate Sealer | 2 pieces | |

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

## Materials prepared by users

**Instruments:**

Microplate reader (440-480 nm, optimum wavelength: 460 nm), 37°C Incubator, Centrifuge, Water bath

**Reagents:**

Double distilled water

**Consumptive material:**

10 KD ultrafiltration tube

## Reagent preparation

① Equilibrate all reagents to room temperature before use.

② Preparation of working solution：

Dissolve one vial of substrate with 2.5 mL of buffer solution, mix well to dissolve. Store at 2-8°C for 7 days protected from light.

③ Preparation of 200 μmol/L standard solution：

Dilute 200 μL of 1 mmol/L standard solution with 800 μL of double distilled water, mix well to dissolve. Store at 2-8°C for 7 days protected from light.

④ The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 200 μmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 40, 80, 100, 120, 140, 160, 200 μmol/L. Reference is as follows:

| Item | ① | ② | ③ | ④ | ⑤ | ⑥ | ⑦ | ⑧ |
|---|---|---|---|---|---|---|---|---|
| **Concentration (μmol/L)** | **0** | **40** | **80** | **100** | **120** | **140** | **160** | **200** |
| **200 μmol/L standard (μL)** | 0 | 40 | 80 | 100 | 120 | 140 | 160 | 200 |
| **Double distilled water (μL)** | 200 | 160 | 120 | 100 | 80 | 60 | 40 | 0 |

## Sample preparation

### ① Sample preparation

**Serum and plasma:** Filter the sample through a 10 KD ultrafiltration tube, centrifuge at 12000×g for 15 min at 4°C and collect the filtrate for detection.

**Tissue sample:**

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL extraction solution with a dounce homogenizer at 4°C.

④ Centrifuge at 10000×g for 10 min 4°C to remove insoluble material. Collect supernatant, then incubate in boiling water bath for 20 min. Centrifuge at 10000×g for 10 min, then take the supernatant and preserve it on ice for detection.

**Cell (adherent or suspension) samples:**

① Harvest the number of cells needed for each assay (initial recommendation = 1×10^6 cells).

② Wash cells with PBS (0.01 M, pH 7.4).

③ Homogenize 1×10^6 cells in 200 μL extraction solution with a ultrasonic cell disruptor at 4°C.

④ Centrifuge at 10000×g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection.

### ② Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

| Sample type | Dilution factor |
|---|---|
| 10% Fresh salmon tissue homogenate | 1 |
| 10% Pelteobagrus fulvidraco tissue homogenate | 1 |

| | |
|---|---|
| 10% Spanish mackerel tissue homogenate | 1 |
| 10% Spoiled Spanish mackerel tissue homogenate | 1 |
| 10% Thunnini tissue homogenate | 3-6 |
| 10% Spoiled Thunnini tissue homogenate | 4-10 |
| 10% Pepper tissue homogenate | 2-8 |
| 10% Grape flesh tissue homogenate | 1 |
| 10% Garlic tissue homogenate | 1 |
| 10% jujube tissue homogenate | 2-8 |
| Rat serum | 1 |
| Mouse serum | 1 |
| Fetal calf serum | 1 |
| 0.2×10^6 Jurkat cell | 1 |
| 0.2×10^6 4T1 cell | 1 |
| 0.2×10^6 HL-60 cell | 1 |
| 0.2×10^6 293T cell | 1 |
| 0.2×10^6 CHO cell | 1 |

Note: The diluent is extraction solution. For the dilution of other sample types, please do pretest to confirm the dilution factor.

## Operating steps

① Standard well: add 20 μL of standards with different concentrations into the standard wells.
Sample well: add 20 μL of sample into the sample wells.
Control well: add 20 μL of sample into the control wells.

② Add 20 μL of chromogenic solution to the each well.

③ Add 100 μL of working solution to standard and sample well. Add 100 μL of buffer solution to control well.

④ Mix fully for 5 s with microplate reader and incubate at 37°C for 40 min protected from light. Measure the OD values of each well at 460 nm with microplate reader.

## Calculation

**The standard curve**:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the absoluted OD value.

3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

**The sample**:

**1. Serum (plasma) sample:**

$$\text{Histamine content (μmol/L)} = \frac{\Delta A - b}{a} \times f$$

**2. Tissue sample:**

$$\text{Histamine content (μmol/kg wet weight)} = \frac{\Delta A - b}{a} \div \frac{m}{V} \times f$$

**3. Cell sample:**

$$\text{Histamine content (μmol/10^9)} = \frac{\Delta A - b}{a} \div \frac{n}{V} \times f$$

**[Note]**

ΔA: Absolute OD ($OD_{Sample}$ - $OD_{Control}$).

m: the weight of tissue, g.

n: The number of cell sample/10^6.

V: The volume of extraction solution in the preparation step of sample, mL.

f: Dilution factor of sample before test.

# Appendix I Performance Characteristics

## 1. Parameter:

### Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

| Parameters | Sample 1 | Sample 2 | Sample 3 |
|---|---|---|---|
| **Mean (μmol/L)** | 25.00 | 50.00 | 80.00 |
| **%CV** | 0.5 | 0.8 | 1.6 |

### Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

| Parameters | Sample 1 | Sample 2 | Sample 3 |
|---|---|---|---|
| **Mean (μmol/L)** | 25.00 | 50.00 | 80.00 |
| **%CV** | 1.5 | 2.3 | 4.2 |

### Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 102%.

| | Standard 1 | Standard 2 | Standard 3 |
|---|---|---|---|
| **Expected Conc. (μmol/L)** | 25 | 50 | 80 |
| **Observed Conc. (μmol/L)** | 24.25 | 51 | 86.2 |
| **Recovery rate (%)** | 97 | 102 | 108 |

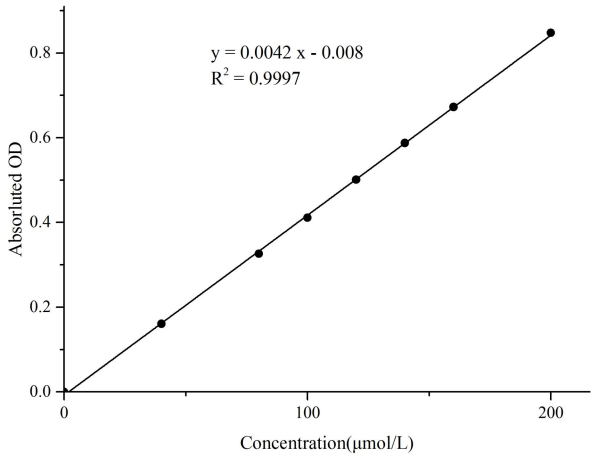
### Sensitivity

The analytical sensitivity of the assay is 2.44 μmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

**2. Standard curve:**

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only:

| **Concentration (μmol/L)** | **0** | **40** | **80** | **100** | **120** | **140** | **160** | **200** |
|---|---|---|---|---|---|---|---|---|
| **OD value** | 0.068 | 0.227 | 0.395 | 0.478 | 0.565 | 0.651 | 0.742 | 0.915 |
| | 0.068 | 0.230 | 0.393 | 0.480 | 0.573 | 0.660 | 0.739 | 0.916 |
| **Average OD** | 0.068 | 0.229 | 0.394 | 0.479 | 0.569 | 0.656 | 0.741 | 0.916 |
| **Absoluted OD** | 0 | 0.161 | 0.326 | 0.411 | 0.501 | 0.588 | 0.673 | 0.848 |



# Appendix II Example Analysis

**Example analysis：**

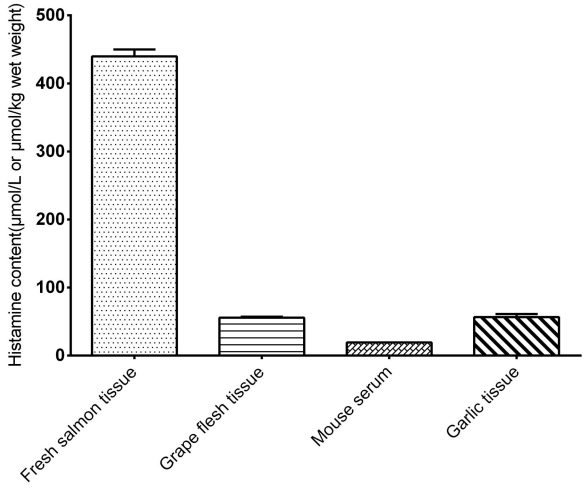
For 10% fresh salmon tissue homogenate, take 20 μL of sample and carry the assay according to the operation table. The results are as follows:

Standard curve: y = 0.0042 x - 0.008, the average OD value of the sample is 0.303, the average OD value of the control is 0.106, and the calculation result is:

Histamine content (μmol/kg wet weight)

= (0.303 – 0.106 + 0.008) ÷ 0.0042 ÷ (0.1 ÷ 0.9) = 439.64 μmol/kg wet weight

Detect 10% fresh salmon tissue homogenate, 10% grape flesh tissue homogenate, mouse serum and 10% garlic tissue homogenate, according to the protocol, the result is as follows：



## Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.