



Histamine Microplate Assay Kit

User Manual

Catalog # CAK1230

(Version 1.3B)

Detection and Quantification of Histamine Content in Tissue
extracts and Other biological fluids Samples.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION.....	2
II. KIT COMPONENTS.....	3
III. MATERIALS REQUIRED BUT NOT PROVIDED.....	3
IV. SAMPLE PREPARATION.....	4
V. ASSAY PROCEDURE.....	5
VI. CALCULATION.....	6
VII. TYPICAL DATA.....	7
VIII. TECHNICAL SUPPORT.....	7
IX. NOTES.....	7

I. INTRODUCTION

Histamine is a biogenic amine naturally present in many foods and body cells in amounts without toxicological significance. It is also a contaminant that mostly found in the Scombridae family fishes such as tuna and mackerel or other seafood products when improperly handled or stored. The consumption of foods containing high level of histamine may lead to an allergy-like food poisoning known as scombroid poisoning.

Histamine Microplate Assay Kit is a sensitive assay for determining Histamine content in various samples. Histamine is hydrolyzed by histamine dehydrogenase. The intensity of product color, measured at 450 nm is directly proportional to histamine concentration in the sample.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	15 ml x 1	4 °C
Enzyme	Powder x 1	-20 °C
Dye Reagent A	Powder x 1	4 °C
Dye Reagent B	1 ml x 1	4 °C
Standard	Powder x 1	4 °C
Technical Manual	1 Manual	

Note:

Dye Reagent A: add 1 ml distilled water to dissolve before use, mix. Store at -20°C for a month.

Enzyme: add 1 ml Reaction Buffer to dissolve before use, mix. Store at -80°C for a month.

Standard: add 1 ml distilled water to dissolve, mix; then add 10 µl solution into 490 µl distilled water, mix. The concentration will be 1 mmol/L. Store at -20°C for a month.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 450 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer

IV. SAMPLE PREPARATION

1. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice; incubate at 60 °C water bath for 30 minutes; centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube for detection.

3. For liquid samples

Detect directly, or dilute with Assay Buffer.

V. ASSAY PROCEDURE

Warm all reagents to room temperature before use.

Add following reagents into the microplate:

Reagent	Sample	Standard	Blank
Reaction Buffer	150 μ l	150 μ l	150 μ l
Sample	20 μ l	--	--
Standard	--	20 μ l	--
Distilled water	--	--	20 μ l
Enzyme	10 μ l	10 μ l	10 μ l
Dye Reagent A	10 μ l	10 μ l	10 μ l
Dye Reagent B	10 μ l	10 μ l	10 μ l
Mix, incubate at room temperature for 10 minutes, measured at 450 nm and record the absorbance.			

Note:

- 1) Perform 2-fold serial dilutions of the top standards to make the standard curve.
- 2) The concentrations can vary over a wide range depending on the different samples.
For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.
- 3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

1. According to the volume of sample

$$\begin{aligned} \text{Histamine } (\mu\text{mol/ml}) &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Blank}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / \\ &\quad V_{\text{Sample}} \\ &= (OD_{\text{Sample}} - OD_{\text{Blank}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) \end{aligned}$$

2. According to the weight of sample

$$\begin{aligned} \text{Histamine } (\mu\text{mol/g}) &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Blank}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / \\ &\quad (W \times V_{\text{Sample}} / V_{\text{Assay}}) \\ &= (OD_{\text{Sample}} - OD_{\text{Blank}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / W \end{aligned}$$

C_{Protein} : the protein concentration, mg/ml;

C_{Standard} : the standard concentration, 1 mmol/L = 1 $\mu\text{mol/ml}$;

W : the weight of sample, g;

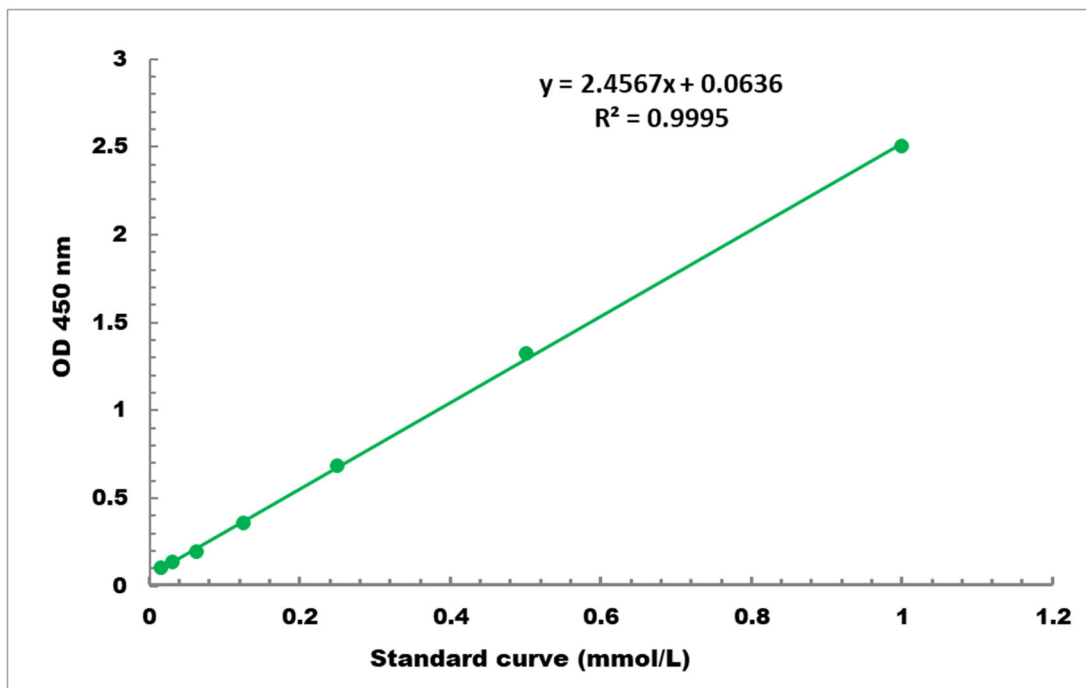
V_{Sample} : the volume of sample, 0.02 ml;

V_{Standard} : the volume of standard, 0.02 ml;

V_{Assay} : the volume of Assay buffer, 1 ml.

VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.01 mmol/L - 1 mmol/L

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES