



Histamine Colorimetric Microplate Assay Kit User Manual

Catalog # CAK1230

(Version 1.4B)

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

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

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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 Colorimetric 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, keep in dark
Dye Reagent B	1 ml x 1	4 °C, keep in dark
Standard	Powder x 1	4 °C
Technical Manual	1 Manual	

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. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. Dissolve in 1 ml distilled water to generate 50 mmol/L of standard stock/top solution, store at 4 °C for 1 month after reconstitution. Then dilute to 1 mmol/L standard top solution by adding 10 μ l stock solution into 490 μ l distilled water. Perform 2-fold serial dilutions of the top standard solution using distilled water to make the standard curve. The concentration of standard curve could be 1/0.5/0.25/0.125/0.0625/ 0.0312/0.0156 mmol/L.

Dye Reagent A: Briefly centrifuge prior to opening. Add 1 ml distilled water to dissolve before use, mix. Keep in dark and store at 4°C for 1 week or -20°C for 1 month.

Enzyme: Briefly centrifuge prior to opening. Add 1 ml Reaction Buffer to dissolve before use, mix. Store at -80°C for 1 month.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. 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.

VI. 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, measure at 450 nm and record the absorbance.			

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**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.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

1. Calculate the sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

$$C = \frac{(OD_{\text{Sample}} - OD_{\text{Blank}}) - \text{Intercept}}{\text{Slope}} \times n (\text{mmol/L})$$

Calculate the initial concentration according to sample preparation procedure.

2. According to one point of the standard OD and concentration

2.1 According to the weight of sample

$$C = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Blank}})}{(OD_{\text{Standard}} - OD_{\text{Blank}}) \times W \times (V_{\text{Sample}} / V_{\text{Assay}})} (\mu\text{mol/g})$$

2.2 According to the volume of sample

$$C = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Blank}})}{(OD_{\text{Standard}} - OD_{\text{Blank}}) \times V_{\text{Sample}}} (\mu\text{mol/ml})$$

Slope: the absorbance slope of standard curve

n: the dilution factor

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

V_{Standard} : the volume of standard in assay procedure, μl

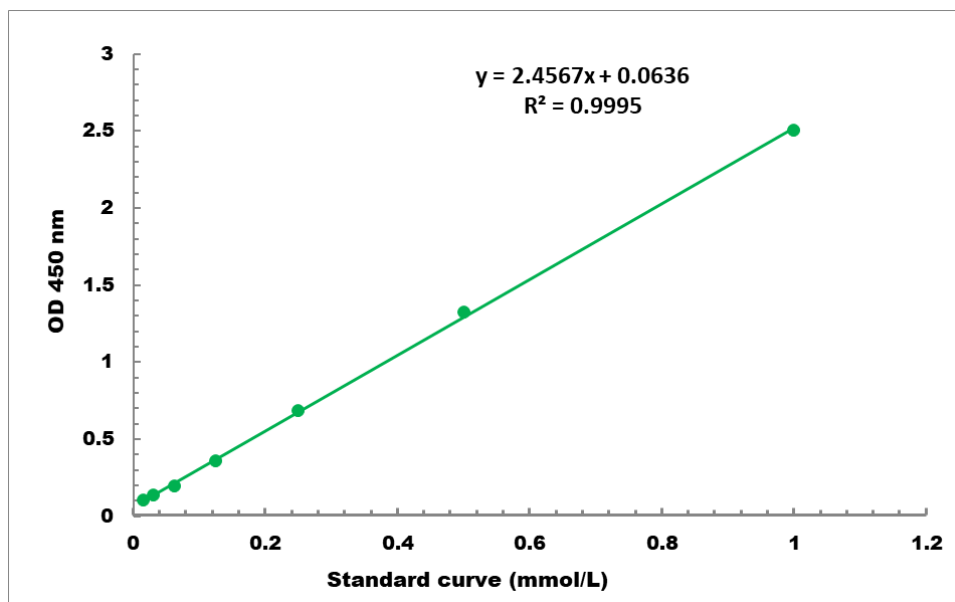
V_{Sample} : the volume of sample in assay procedure, μl

V_{Assay} : the volume of Assay Buffer, μl

W: the weight of sample, g

VIII. TYPICAL DATA

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



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