Histamine Dehydrogenase

Microplate Assay Kit

User Manual

Catalog # CAK1307

(Version 1.1A)

Detection and Quantification of Histamine Dehydrogenase (HDH) Activity in Tissue extracts, Cell lysate, Cell culture mediaand Other biological fluids Samples.

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

I. INTRODUCTION

Histamine dehydrogenase is a homodimeric enzyme and catalyzes oxidative deamination of histamine.Histamine dehydrogenase can be isolated from cultures of Nocardiodes simplex grown on histamine as the sole nitrogen source. The enzyme is a homodimer of a ~76kDa subunit responsible for catalyzing the oxidative deamination of histamine to give imidazole acetaldehyde (Scheme 1), where the enzyme exhibits remarkable selectivity for histamine, thus showing great potential for use in biosensors. HADH was originally classified as a quinone-containing amine dehydrogenase. Later studies revealed that HADH is a homologue of TMADH, and dimethylamine dehydrogenase (DMADH) from M. methylotrophus shares 40% sequence identity and 56% similarity with both proteins.

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

II.KIT COMPONENTS

Component	Volume	Storage
96-WellMicroplate	1 plate	
Assay Buffer	30 mlx 4	4 °C
Reaction Buffer	15 mlx 1	4 °C
Substrate	Powder x 1	4 °C
Dye ReagentA	Powder x 1	4 °C
Dye Reagent B	1 ml x 1	4 °C
Standard	Powder x 1	4 °C
Positive Control	Powder x 1	-20 °C
Technical Manual	1 Manual	

Note:

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

Substrate:add 1 mlReaction Buffer to dissolve before use, mix.Store at -20°C for a month.

Standard: add 1 mldistilled water to dissolve, mix; the concentration will be 1mmol/L.Store at -20°C for a month.

Positive Control:add 0.5 ml Assay Buffer to dissolve before use; store at -80 °C for a month after reconstitution.

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 cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 mlAssay buffer for 5 ×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s,repeat 30 times); centrifuged at 8000g 4°C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2.For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 mlAssay buffer on ice, centrifuged at 8000g 4°C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3.For liquidsamples

Liquid samples can be used directly.

V. ASSAY PROCEDURE

Warm all regents to room temperature before use.

Add following reagents intothe microplate:

Reagent	Sample	Control	Standard	Blank	Positive Control
Substrate	170 µl	170 µl	170 µl	170 µl	170 µl
Sample	10µl	--	--	--	--
Assay Buffer	--	10 µl	--	--	--
Positive Control	--	--	--	--	10 µl
Standard	--	--	10 µl	--	--
Distilled water	--	--	--	10 µl	--
Dye Reagent A	10 µl	10 µl	10 µl	10 µl	10 µl
Dye Reagent B	10 µl	10 µl	10 µl	10 µl	10 µl
Mix, incubate at room temperature for 5 minutes, measured at 450 nm and recordthe 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

Unit Definition: One unit of HDH activity is defined as the amount of enzyme whichreduces 1 μmol of H^+ per min at 37°C.

1. According to the volume of sample

$$HDH\ (\mu mol/ml) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} / T$$

$$= 0.2 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank})$$

2. According to the weight of sample

$$HDH\ (\mu mol/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (W \times V_{Sample} / V_{Assay}) / T$$

$$= 0.2 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / W$$

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

$C_{Standard}$: the standard concentration, 1mmol/L = 1μmol/ml;

W: the weight of sample, g;

V_{Sample}: the volume of sample, 0.01 ml;

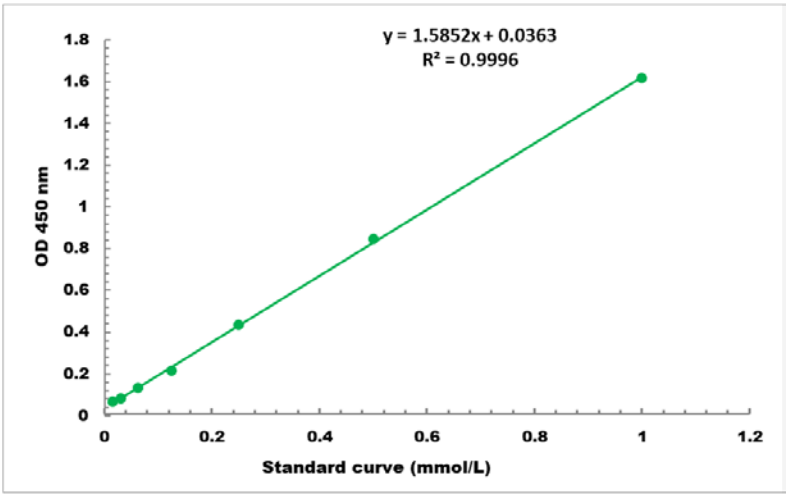
$V_{Standard}$: the volume of standard, 0.01 ml;

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

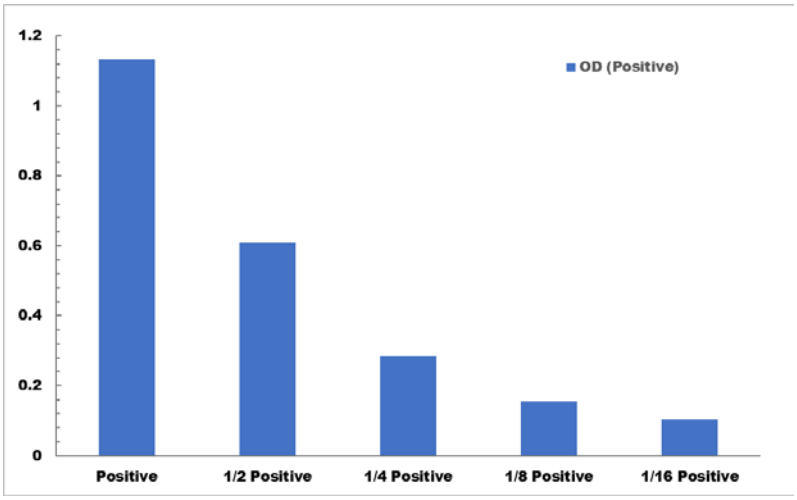
T: the reaction time, 5 minutes.

VII. 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



Positive Control reaction in 96-well plate assay with decreasing the concentration

VIII. TECHNICAL SUPPORT

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

IX. NOTES