Histamine Dehydrogenase Activity Microplate Assay Kit

Instructions for Use | Cat. No.: ARS6871

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

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

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

2. 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	-

3. 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

4. Reagent Preparation

Standard: Briefly centrifuge prior to opening. Dissolve in 1 mL distilled water to generate 2 mmol/ L of standard stock solution , store at 4°C for 1 day or -20°C for3days after reconstitution. Then dilute to 1 mmol/L standard top solution by adding 0.5 mL stock solution into 0. 5 mL 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/ 0.0312/0.0156mmol/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.

Substrate: Briefly centrifuge prior to opening. Add 1 mL Reaction Buffer to dissolve before use , mix.Store at 4°C for 1-2 weeks or -20°C for 3 -4months after reconstitution.

Positive Control: Briefly centrifuge prior to opening. Add 0.5 mL Assay Buffer to dissolve before use. Store at -80°C for 1 month after reconstitution.

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

5. Sample Preparation

1. For cell and bacteria samples Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 mL Assay 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 mL Assay Buffer on ice, centrifuged at 8000 g 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.

6. Assay Procedure

Warm all regents to room temperature before use . Add following reagents in to the 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:

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

**For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more samples into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

7. Calculation

Unit Definition:

One unit of HDH activity is defined as the amount of enzyme

which reduces 1 μmol of H^+ per min at 37°C.

1. According to the slope of the standard curve

$$\text{Activity} = \frac{(OD_{Sample} - OD_{Control}) - \text{Intercept}}{\text{Slope} \times T} \times n \ (U/mL)$$

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

2.1 According to the protein concentration of sample

$$\text{Activity} = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control})}{(OD_{Standard} - OD_{Blank}) \times V_{Sample} \times C_{Protein} \times T} \ (U/mg)$$

2.2According to the quantity of cells or bacteria

$$\text{Activity} = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control})}{(OD_{Standard} - OD_{Blank}) \times N \times (V_{Sample} / V_{Assay}) \times T} \ (U/10^4)$$

2.3 According to the weight of sample

$$\text{Activity} = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control})}{(OD_{Standard} - OD_{Blank}) \times W \times (V_{Sample} / V_{Assay}) \times T} \ (U/g)$$

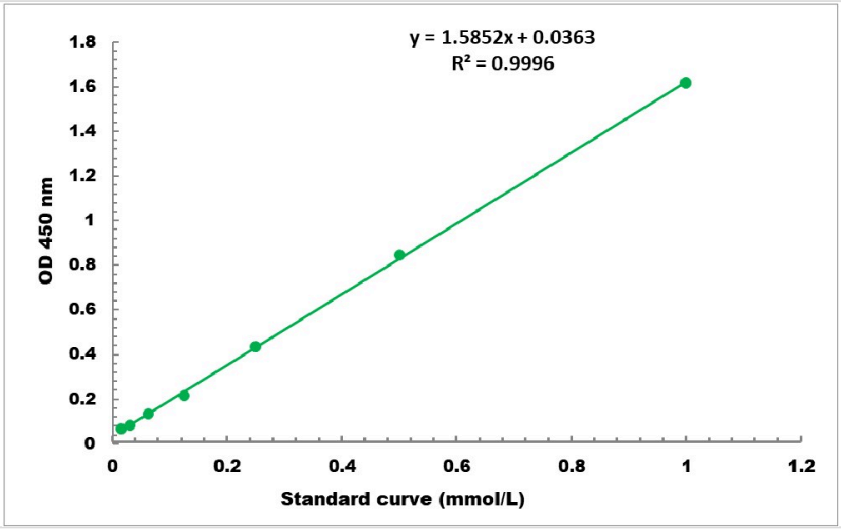
2.4 According to the volume of sample

$$\text{Activity} = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control})}{(OD_{Standard} - OD_{Blank}) \times V_{Sample} \times T} \ (U/mL)$$

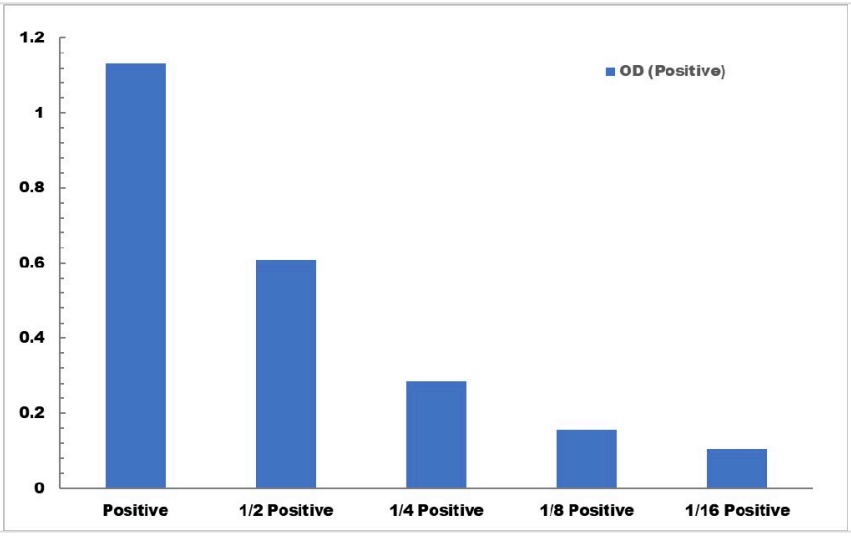
Item	Definition
Slope	the absorbance slope of standard curve
n	the dilution factor
$C_{Protein}$	the protein concentration of sample, mg/mL
W	the weight of total sample , g
N	the quantity of total cell or bacteria sample, 10^4
$C_{Standard}$	the concentration of standard, μmol/mL
$V_{Standard}$	the volume of standard in assay procedure, mL
V_{Sample}	the volume of sample in assay procedure, mL
V_{Assay}	the volume of Assay Buffer in sample preparation , mL
T	the reaction time , minute

8. Typical Data

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



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



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