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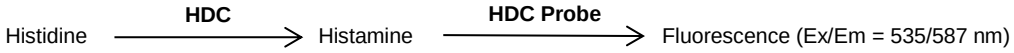
Histidine Decarboxylase Activity Assay Kit (Fluorometric)

rev 04/21

(Catalog # K2082-100; 100 assays, Store kit at -20 °C)

I. Introduction:

Histidine decarboxylase (HDC, 4.1.1.22) is an enzyme that catalyzes the decarboxylation of histidine to form histamine. Histamine is a very critical biogenic amine in mammals and plays a key regulatory role in neurotransmission, inflammamation, gastric acid secretion, allergy and immune response. HDC activity is the primary source of histamine in most mammals and eukaryotes and this metabolite cannot be produced by any other known enzyme. In humans, HDC is primarily expressed in mast cells and basophil granulocytes. But it is also present in non-mast cells including brain and kidney. Besides mammals, HDC is abundant in histamine-producing bacteria such as *Photobacterium phosphoreum* and *Raoultella planticola*, which can cause histamine poisoning in foods such as fish and cheese. **BioVison's Histidine Decarboxylase Activity Assay Kit** is designed to determine the HDC activity in various sample types. In this assay, HDC converts histidine to produce histamine, which then reacts with the HDC probe to generate a strong fluorescent signal measured at Ex/Em = 535/587 nm. The fluorescent signal is directly proportional to the HDC activity in the samples. The assay is simple, fast and is high throughput adaptable. The kit can detect as low as 0.01 mU of HDC activity in samples under assay conditions.



II. Application:

- To measure histidine decarboxylase activity in various sample types.

III. Sample Types:

- Purified Protein
- Bacterial lysates

IV. Kit Contents:

Components	K2082-100	Cap Code	Part Number
HDC Assay Buffer	25 ml	WM	K2082-100-1
Histidine	1.2 ml	Orange	K2082-100-2
HDC Enzyme Mix	1 vial	Purple	K2082-100-3
HDC Developer	1 vial	Green	K2082-100-4
HDC Probe	200 µl	Red	K2082-100-5
Histamine Standard	100 µl	Yellow	K2082-100-6
HDC Positive Control	1 vial	Blue	K2082-100-7

V. User Supplied Reagents and Equipment:

- 96-well white plate with flat bottom
- Multi-well spectrophotometer
- PBS
- Phosphate buffer
- β-mercaptoethanol
- Glycerol

VI. Storage Conditions and Reagent Preparation:

Store the kit at -20 °C. The kit components are stable for one year when stored as recommended. Briefly centrifuge all small vials at a low speed prior to opening. Read the entire protocol before performing the experiment.

- **HDC Assay Buffer, Histidine and HDC Probe:** Ready to use as supplied. Thaw and bring to room temperature (RT) before use. Store at -20 °C.
- **HDC Enzyme Mix and HDC Developer:** Reconstitute each vial with 220 µl of HDC Assay Buffer. Divided into aliquots and store at -20 °C. Use within 2 months.
- **Histamine Standard (50 mM):** Thaw and bring to RT before use. Store at -20 °C.
- **HDC Positive Control:** Reconstitute the vial with 200 µl of 20 mM phosphate buffer containing 5 mM β-mercaptoethanol and 20% glycerol (not supplied). Aliquot and avoid freeze/thaw. Store at -20 °C.

VII. HDC Activity Assay Protocol:

1. Sample Preparation: For bacterial lysates, grow 50 ml of bacteria in any suitable growth media (i.e. LB or any other media) overnight at 37 °C. After incubation, harvest the cells by centrifuging at 10,000 x *g* for 20 min. Add 5 ml ice-cold PBS per 1 gram of wet cell pellet. Sonicate the cells for 5 min on ice and centrifuge at 10,000 x *g* and 4 °C for 20 min. Transfer the clear supernatant to a new eppendorf tube. For each sample type, add 2-10 µl of the supernatant into the desired well(s) of a 96-well white plate with flat bottom labeled as **Sample** and adjust the volume to 50 µl/well using HDC Assay Buffer. Add 50 µl of HDC Assay Buffer into another well labeled as **Background Control**. For **Positive Control** well, add 5 µl of reconstituted HDC Positive Control into a designated well(s) and adjust the volume to 50 µl/well using HDC Assay Buffer.

Notes:

a) For Unknown Samples, we recommend doing a pilot experiment and testing several doses to ensure that the readings are within the linear range of the Standard Curve.

b) For samples exhibiting significant background, prepare parallel sample well(s) designated as **Sample Background**.

2. Histamine Standard Curve Preparation: Mix 10 µl of 50 mM Histamine Standard with 990 µl of dH_2O to prepare 0.5 mM Histamine Standard. Then mix 50 µl of the 0.5 mM Histamine Standard with 950 µl of dH_2O to prepare 25 µM Histamine Standard. Add 0, 2, 4, 6, 8 and 10 µl of 25 µM Histamine Standard into the desired wells of a 96-well white plate to generate 0, 50, 100, 150, 200, 250 pmole Histamine Standard/well. Adjust the volume of all Standard wells to 50 µl/well with HDC Assay Buffer.

3. Reaction Mix Preparation: Mix enough reagents for the number of assays to be performed. Prepare 50 µl of Reaction Mix and 50 µl of Background Control Mix as indicated below:

	Reaction Mix	Sample Background Mix
HDC Assay Buffer	35 µl	45 µl
Histidine	10 µl	--
HDC Enzyme Mix	2 µl	2 µl
HDC Developer	2 µl	2 µl
HDC Probe	1 µl	1 µl

Mix well. Add 50 µl of Reaction Mix to wells containing **Standards**, **Samples**, **Background Control** and **Positive Control**. Add 50 µl of Sample Background Mix to the **Sample Background** well(s).

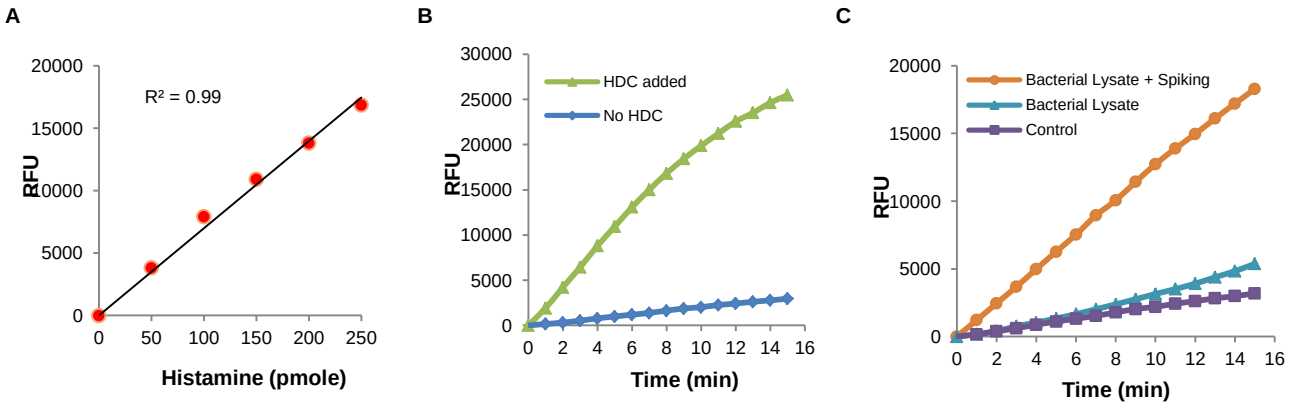
4. Measurement: Measure the fluorescence of all wells at Ex/Em= 535/587 nm in kinetic mode at 25 °C for 15-30 min.

5. Calculation: Subtract the 0 Standard reading from all Standard readings. Plot the Histamine Standard Curve. If the sample background is significant, subtract the **Sample Background** reading from its paired sample reading to get the corrected Sample readings. Choose any two time points within the linear portion of the Standard Curve (t_1 and t_2) for each Sample type. Apply the corrected Sample readings to the Histamine Standard Curve to get B pmole of Histamine generated during the reaction time ($\Delta t = t_2 - t_1$). Calculate the HDC activity of the Samples using the following equation:

HDC Activity = B x D / (Δt x V) = pmol/min/ml (mU/ml)

Where: **B** is the amount of Histamine from the Standard Curve (in pmole)
Δt is the reaction time (in min)
V is the sample volume added to the well (in ml)
D is the sample dilution factor (if applicable, D = 1 for Undiluted Samples)

Unit Definition: One unit is 1 µmole of Histamine generated by HDC per min at pH 6.0 and 25 °C.



Figures. A. Histamine Standard Curve. **B.** Reaction curve of HDC activity. **C.** HDC activity in bacterial lysates before and after spiking with HDC. >95% recovery was observed. Assay was performed according to assay protocol and showed >95% recovery.

VIII. Related Products:

Histamine Assay Kit (Fluorometric) (K386)
Total Polyamine Assay Kit (Fluorometric) (K475)
Histamine Assay Kit (Colorimetric) (K506)
Methionine Assay Kit (Fluorometric) (K442)
Diamine Oxidase Activity Assay Kit (Fluorometric) (K496)
Protein Carbonyl Content Assay Kit (Fluorometric) (K563)

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