AB308324 – Histidine Decarboxylase Activity Assay Kit (Fluorometric)

To measure histidine decarboxylase activity in various sample types..
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: abcam website

Storage and Stability

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.

Materials Supplied

Item	Quantity	Storage Condition
HDC Assay Buffer	25 ml	-20°C
Histidine	1.2 ml	-20°C
HDC Enzyme Mix	1 vial	-20°C
HDC Developer	1 vial	-20°C
HDC Probe (Avoid light)	200 µl	-20°C
Histamine Standard	100 µl	-20°C
HDC Positive Control	1 vial	-20°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

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

Reagent Preparation

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.

HDC Activity Assay Protocol:

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.

Δ Note:

1. *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.*
2. *For samples exhibiting significant background, prepare parallel sample well(s) designated as Sample Background.*

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.

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).

Measurement:

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

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:

$$\text{HDC Activity} = B \times D / (t \times V) = \text{pmol/min/ml (mU/ml)}$$

Where: **B** is the amount of Histamine from the Standard Curve (in pmole)
t 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.

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)