Product Manual

Histidine Assay Kit

Catalog Number

MET-5194 **100 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Histidine is an essential amino acid (obtained from the diet) that is used in protein biosynthesis. Histidine is a positively charged amino acid due to its partially protonated imidazole side chain which acts as a ligand to bind various metals including iron, nickel, zinc, and cobalt. In addition, histidine is a precursor for histamine, an amine produced in the body necessary for combating inflammation. The enzyme histidine ammonia-lyase produces ammonia and urocanic acid from the substrate histidine and deficiency mutants for this activity is present in the rare metabolic disorder histidinemia. Histidine can be converted to the dipeptide carnosine found in skeletal muscle. Histidine can also be converted by certain methyltransferase enzymes to 3-methylhistidine, which is a skeletal muscle damage marker.

Cell Biolabs' Histidine Assay Kit is a simple assay for measuring histidine levels in biological samples. Total histidine levels may be quantified in a wide range of biological samples including serum and plasma. The kit has a detection sensitivity limit of 7.8 µM histidine. Each kit provides sufficient reagents to perform up to 100 assays*, including standard curve and unknown samples.

Note: Each sample replicate requires 2 assays, one treated with Histidine Decarboxylase (+HDC) and one without (-HDC). The histidine level is calculated from the difference in OD 450 readings from the 2 wells.

Assay Principle

The Histidine Assay Kit is a sensitive quantitative colorimetric assay for histidine. Histidine Decarboxylase converts histidine to histamine. A Reaction Mix containing WST-1, an electron mediator, and Histamine Dehydrogenase (HDH) is then added. During a brief incubation, the WST-1 is converted to the formazan form (Figure 1) and the absorbance of the plate is read at 450 nm. A standard curve is generated from known concentrations of the histidine standard, and samples are then compared to the standard curve to determine the histidine content.

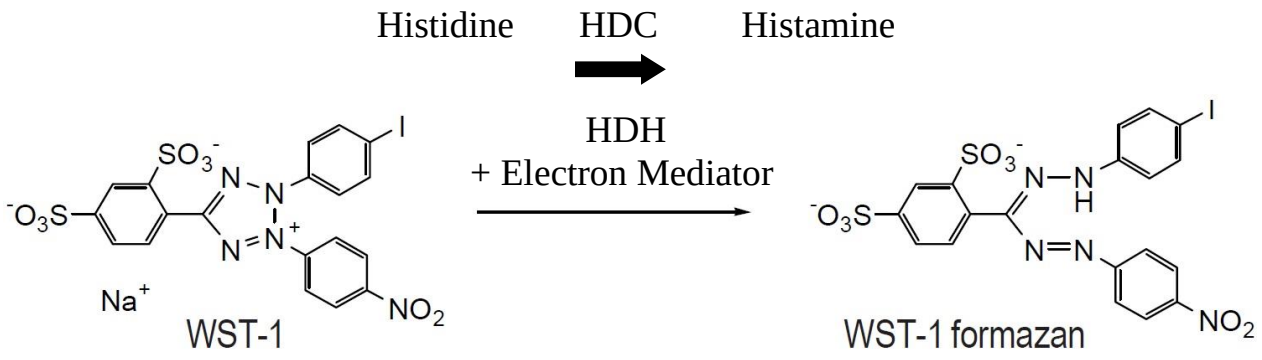

Figure 1. Assay Principle.

Related Products

1. MET-5054: L-Amino Acid Assay Kit
2. MET-5056: Branched Chain Amino Acid Assay Kit
3. MET-5071: Taurine Assay Kit

4. MET-5136: D-Amino Acid Kit (Colorimetric)
5. MET-5158: Methionine Assay Kit

Kit Components

Box 1 (shipped on blue ice packs)

1. 10X Assay Buffer (Part No. 51942A): One 30 mL bottle.
2. 10X Reaction Buffer (Part No. 51943A): One 30 mL bottle.

Box 2 (shipped on blue ice packs)

1. L-Histidine Standard (Part No. 51941C): One 250 µL vial at 10 mM.
2. Histidine Decarboxylase (Part No. 51944D): One 500 µL vial.
3. Histamine Dehydrogenase (1000X) (Part No. 51945D): One 20 µL vial at 17 U/mL
   Note: One unit is defined as the amount of enzyme that produces 1 µmol of 4-imidazolylacetoaldehyde per minute at 37 ºC and pH 9.0.
4. 10X Colorimetric Probe (Part No. 51946D): Two 1 mL amber vials

Materials Not Supplied

1. Spectrophotometric microplate reader capable of reading absorbance at 450 nm (620 nm as optional reference wavelength)
2. 10 kD cutoff centrifugal filter units

Storage

Upon receipt, store the 10X Assay Buffer and 10X Reaction Buffer at room temperature. Store all remaining components at -80ºC. Avoid multiple freeze/thaw cycles. The Colorimetric Probe is light sensitive and must be stored accordingly.

Preparation of Reagents

Note: All reagents must be brought to room temperature prior to use.

- 1X Assay Buffer: Dilute the stock 10X Assay Buffer 1:10 with deionized water for a 1X solution. Stir or vortex to homogeneity.
- 1X Reaction Buffer: Dilute the stock 10X Reaction Buffer 1:10 with deionized water for a 1X solution. Stir or vortex to homogeneity.
- Reaction Mix: Prepare a Reaction Mix by diluting the Colorimetric Probe 1:10 and the Histamine Dehydrogenase 1:1000 in 1X Reaction Buffer. For example, for 20 assays, add 400 µL of the 10X Colorimetric Probe and 4 µL of the Histamine Dehydrogenase to 3596 µL of 1X Reaction Buffer.

  Note: Prepare only enough for immediate use by scaling the above example proportionally.

Preparation of Samples

Note: Maintain pH between 7 and 8 for optimal working conditions as the Colorimetric Probe is unstable at high pH (>8.5).

- Cell lysates: Resuspend cells in 1X Assay Buffer. Homogenize or sonicate the cells on ice. Centrifuge 10,000 x g for 10 minutes at 4°C to remove debris. Collect the supernatant and filter the solution with a 10kD cutoff centrifugal filter unit to deproteinate the sample. Collect flow through. The flow through may be assayed undiluted or diluted as necessary in 1X Assay Buffer.

- Tissue lysates: Sonicate or homogenize tissue sample in 1X Assay Buffer and centrifuge at 10,000 x g for 10 minutes at 4°C. Collect the supernatant and filter the solution with a 10kD cutoff centrifugal filter unit to deproteinate the sample. Collect flow through. The flow through may be assayed directly or diluted as necessary in 1X Assay Buffer.

- Serum or Plasma: Deproteinate the sample by running it through a 10 kD centrifugal filter unit and collecting the flow through. To remove insoluble particles, centrifuge at 10,000 rpm for 5 min. Dilute the supernatant as necessary into 1X Assay Buffer just prior to performing the assay.

- Urine: To remove insoluble particles, centrifuge at 10000 x g for 10 min at 4°C. Collect the supernatant and filter the solution with a 10kD cutoff centrifugal filter unit to deproteinate the sample. Collect flow through. The flow through can be assayed directly or diluted as necessary in 1X Assay Buffer.

Preparation of Standard Curve

Prepare fresh Histidine standards before use by diluting in distilled water according to Table 1 below.

Standard Tubes	10 mM L-Histidine Standard (µL)	Distilled Water (µL)	L-Histidine (µM)
1	25	475	500
2	250 of Tube #1	250	250
3	250 of Tube #2	250	125
4	250 of Tube #3	250	62.5
5	250 of Tube #4	250	31.5
6	250 of Tube #5	250	15.6
7	250 of Tube #6	250	7.8
8	0	250	0

Table 1. Preparation of L-Histidine Standards

Assay Protocol

1. Prepare and mix all reagents thoroughly before use. Each sample, including unknowns and standards, should be assayed in duplicate or triplicate.

   Note: Each unknown sample replicate requires two paired wells, one to be treated with Histidine Decarboxylase (+HDC) and one without the enzyme (-HDC) to measure endogenous background.

2. Add 50 µL of each Histidine Standard or unknown sample into wells of a 96-well microtiter plate.

3. Add 5 µL of Histidine Decarboxylase *(+HDC)* to the standards and to one half of the paired sample wells, and mix the well contents thoroughly.
4. Add 5 µL of 1X Assay Buffer *(-HDC)* to the other half of the paired sample wells.
5. Mix the well contents thoroughly and incubate for 60 minutes at 37°C protected from light.
6. Add 200 µL of Reaction Mix per well.
7. Incubate for 15-60 minutes at 37°C protected from light.

   Note: This assay is continuous (not terminated) and therefore may be measured at multiple time points to follow the reaction kinetics.
8. Read absorbance of each well on a microplate reader using 450 nm as the primary wave length.

Calculation of Results

1. Determine the average absorbance values for each sample, control, and standard.
2. Subtract the average zero standard value from itself and all standard values.
3. Graph the standard curve (see Figure 2).
4. Subtract the sample well values without histidine decarboxylase (-HDC) from the sample well values containing enzyme (+HDC) to obtain the difference. The absorbance difference is due to the enzyme HDC activity:

$$\Delta A = A_{(+HDC)} - A_{(-HDC)}$$

5. Compare the change in absorbance ΔA of each sample to the standard curve to determine and extrapolate the quantity of histidine present in the sample. Only use values within the range of the standard curve.
6. To calculate the final histidine concentration in the sample, take into account any prior dilutions performed.

Example of Results

The following figures demonstrate typical Histidine Assay results. One should use the data below for reference only. This data should not be used to interpret actual results.

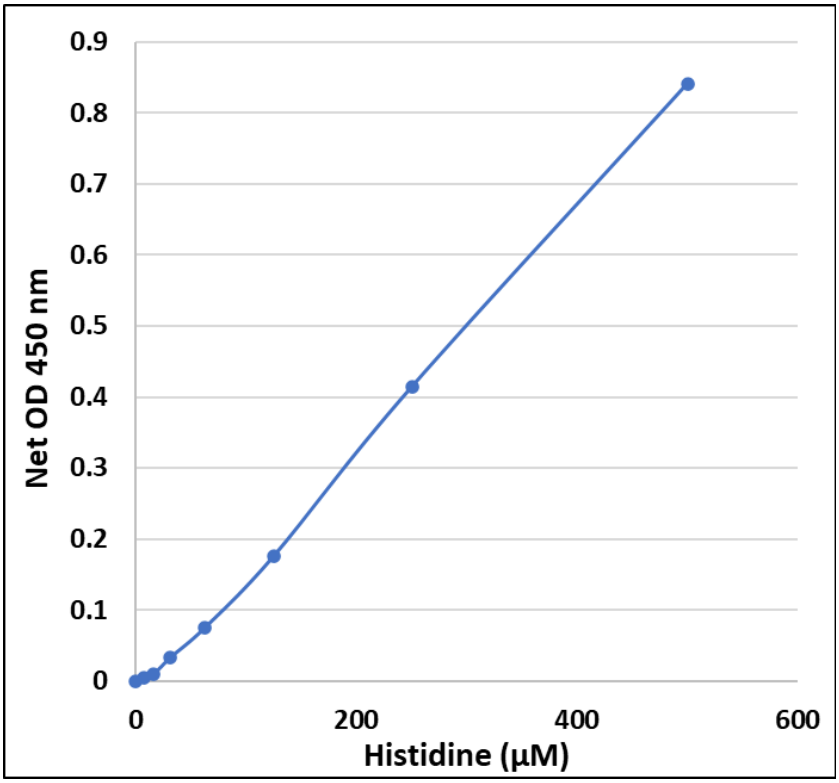

Figure 2. Histidine Standard Curve.

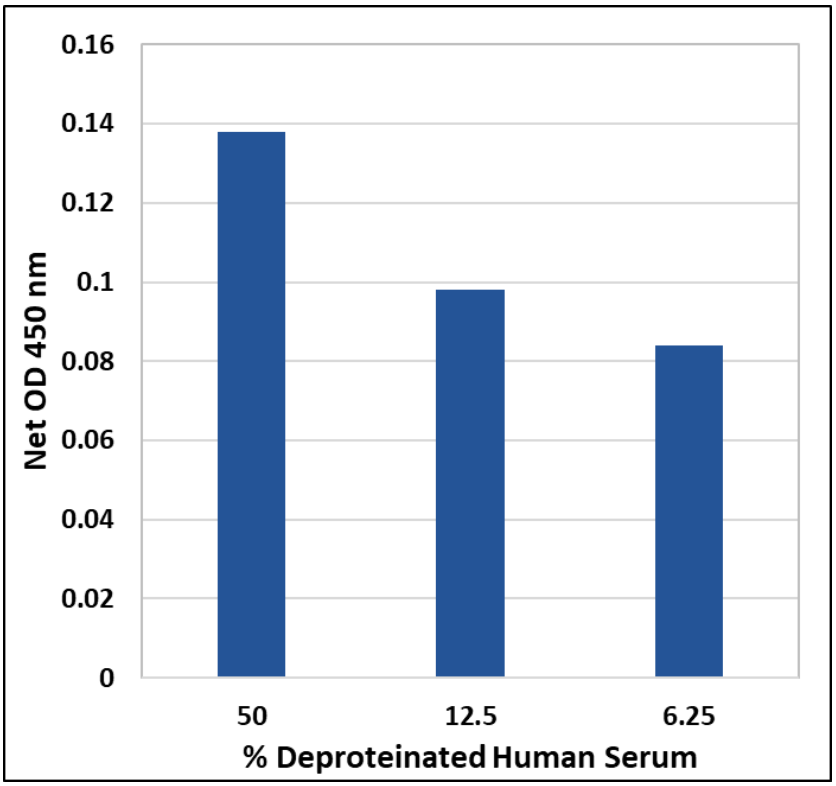

Figure 3. Detection of free histidine in human serum. Human serum was deproteinated according to the Preparation of Samples section above.

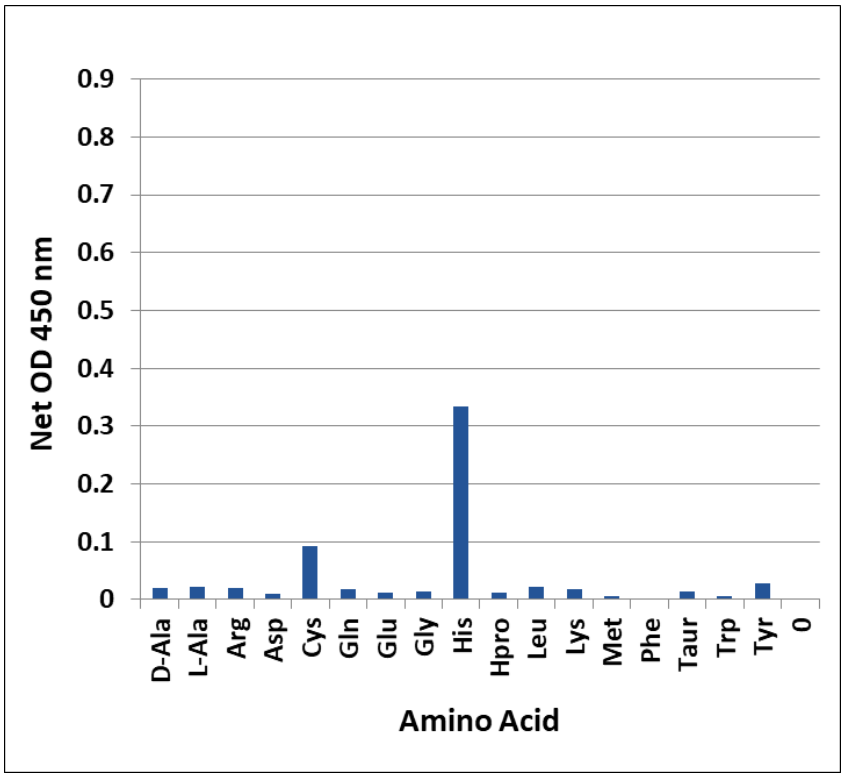

Figure 4. Specificity of Histidine Assay Kit. Reactions were performed in the presence of 250 µM D-Alanine (D-Ala), L-Alanine (L-Ala), Arginine (Arg), Asparate (Asp), Cysteine (Cys), Glutamine (Gln), Glutamate (Glu), Glycine (Gly), Histidine (His), Hydroxyproline (Hpro), Leucine (Leu), Lysine (Lys), Methionine (Met), Phenylalanine (Phe), Taurine (Taur), Tryptophan (Trp), Tyrosine (Tyr), or no Amino Acid (0).

References

1. Kopple JD and Swendseid ME (1975). *J. Clin. Investigation.* **55**: 881–891.
2. Bornhorst JA and Falke JJ (2000). *Applic. of Chim. Genes Hybr. Proteins Part A: Gene Expr and Prot. Purif. Meth. in Enzymol*. **326**:245–254.
3. Watly J, Simonovsky E, Barbosa N, Spodzieja M, Wieczorek R, Rodziewicz-Motowidlo S, Miller Y, and Kozlowski H (2015). *Inorganic Chemistry*. **54**: 7692–7702.
4. Andersen HH, Elberling J, and Arendt-Nielsen L (2015). *Acta Dermato-Venereologica*. **95**: 771–777.
5. Derave W, Everaert I, Beeckman S, and Baguet Au (2010). "Muscle carnosine metabolism and beta-alanine supplementation in relation to exercise and training". Sports Medicine. 40 (3): 247–263.

Warranty

In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

Contact Information

Cell Biolabs, Inc.
5628 Copley Drive
San Diego, CA 92111
Worldwide: +1 858-271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com