Northwest
Life Science Specialties, LLC

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NWLSS™ Histamine ELISA

Product NWK-HIS01 (96 wells)
For Research Use Only

Simple assay kit for quantitative measurement of histamine in multiple species and sample types.

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Introduction:

Histamine is a heterocyclic primary amine derived from the decarboxylation of the amino acid histidine. It is a mediator of inflammation closely associated with the initial phase of immediate hypersensitivity response (anaphylaxis). Histamine is synthesized by the enzyme histidine decarboxylase and is present in most cells, but typically stored in metachromatic granules of basophils and mast cells (granulocytes). Histamine in the intracellular granules is bound to proteins and inactive until it is released from the cells. Tissue bound mast cells in the skin and nasal mucosa can respond to incoming allergens by releasing histamine which manifests as erythema (redness of skin) and rhinitis (irritation and inflammation) response respectively. In various research areas, it is important to study in-vitro histamine release from peripheral blood basophils. When whole blood from a sensitized subject is exposed to a specific allergen, basophils may respond to the allergen by releasing histamine into the incubation mixture. Using a whole blood sample then, one can assess ex vivo response to a sensitizing antigen as a function of histamine released from the basophils. Other than histamine being an important mediator of immune hypersensitivity, histamine is also found in decaying fish meat, especially that of scombroid fish such as tuna. For this reason histamine is called "Scombrotoxin". Histamine can also be found in wine and cheese.

Histamine can be detected using HPLC with a fluorimetric detector or by radioimmunoassay, and also by enzyme immunoassay (ELISA) which is the methodology of the NWLSS™ Histamine ELISA kit. One attractive feature of studying histamine release using ELISA is that one can treat whole blood with various stimulants to activate cells then measure the amount of histamine released. Histamine content of human whole blood can range from 20 to 200 ng/mL. Normal plasma levels of histamine are typically less than 1 ng/mL whereas 3-7 ng/mL can be found in plasma from subjects exhibiting an allergic response. Animal and fish tissues contain 1-100 µg/g tissue.

Intended Use:
The NWLSS™ Histamine ELISA kit is intended for use in quantifying the concentration of Histamine in biological samples from multiple species and sample types.

Test Principle:
The NWLSS™ Histamine ELISA is a competitive direct enzyme linked immunosorbent assay wherein wells are pre-coated with an anti-histamine monoclonal antibody. Samples or standards are added to the antibody-coated microplate. An enzyme conjugate (Histamine labeled with HRP (horseradish peroxidase) is added to the wells and allowed to compete with samples and standards for binding to the stationary (plate-bound) anti-histamine antibody. After sufficient incubation time, the plate is washed thoroughly to remove all unbound sample, standard and histamine-HRP conjugate. Histamine-HRP conjugate is then detected by the addition of TMB substrate to produce a measurable blue coloration. The plate is then read using a standard 96 well reader with 650 nm capability. Sample histamine content is inversely proportional to the amount of color in each well and can be quantified by direct comparison to the standard curve as generated in the assay.

General Specifications:

Format::	96 wells	
Number of tests:	Triplicate =	29
	Duplicate =	45
Specificity:	Histamine	
Sensitivity:	LLD = 2.5 ng/mL	

Kit Contents:

Microtiter Plate:	Precoated with histamine antibody	96 Wells
Standards:	6 levels of histamine standard (500 uL ea)	6 vials
Conjugate:	Histamine-HRP (Horseradish Peroxidase) Conjugate	1 X 6 mL
Substrate:	3,3', 5,5' Tetramethylbenzidine (TMB)	1 X 20 mL
25X Wash Buffer:	Concentrated Wash Buffer	1 X 30 mL
Dilution Buffer:	10 mM Phosphate buffered saline (PBS) Dry powder to make one liter of solution.	1 X Pouch

Required Materials Not Provided:
Adjustable pipettes and disposable tips
A multi-channel or repeater pipette (recommended)
Labware to dilute Wash Buffer and Mix Dilution Buffer.
Plastic film to cover plate during incubation
Deionized water
1 N HCl (Optional) if user wishes to stop reaction and read at 450 nM.

Required Instrumentation:
Microplate reader with 650 nm capability.

Warnings, Limitations, Precautions:
Glassware:
Histamine may adhere to glass. Therefore, use of glassware should be avoided during sample processing.

pH
The samples to be analyzed with this kit must have a pH of 6-8. Samples that are either too acidic or alkaline can be adjusted using 0.1 N HCl or NaOH.

Storage Instructions:
Upon receipt, store the reagents at 2-8°C. Do not use components beyond the expiration date printed on the label.

All reagents should be brought to room temperature (18-25°C) prior to use.

Assay Preparation
Plate Reader Setup Recommendations

Wavelength:	650 nm if no stop solution added. 450 nM if using optional stop solution
Mode:	Endpoint
Shaker:	On (If Available)
Curve Fit:	Linear or 4-Parameter depending on curve shape obtained.

Reagent Preparation:
Bring all reagents, plate wells to be used samples and calibrators to room temperature (20-25°C) before use.

Wash Buffer:
Prepare the necessary volume of wash buffer by mixing 1 part **25X Wash Buffer** with 24 parts Deionized water. Label as **Working Wash Buffer.**

Standards:
6 standard concentrations are provided ready-to-use.

Histamine-Horseradish Peroxidase Conjugate:
Histamine-HRP Conjugate is provided ready-to-use.

TMB Substrate:
TMB Substrate is provided ready-to-use.

Dilution Buffer:
Mix entire contents of foil pouch in 1 liter deionized water. Label as **"Dilution Buffer"**

Sample Handling/Preparation:
Typically, plasma, tissue homogenates, cell extracts and cell/tissue culture media are capable of being tested using this assay. However, it is beyond the scope of this product insert to describe sample processing in detail for each case.

Since the amount of histamine in different species and sample types differs greatly, it is recommended that researchers conduct preliminary tests to determine the optimal dilution for their specific sample type and model system.

However, general guidelines are provided below for representative sample types:

Plasma:
Normal plasma levels of histamine are less than 1 ng/mL whereas 3-7 ng/mL can found in humans and animals exhibiting an allergic reaction.

Whole Blood:
Normal human whole blood histamine levels of 20 to 200 ng/mL have been reported.

Urine:
Normal human urine levels of 4-54 ng/mL have been reported.

Assay Protocol:

Standard Procedure

1. Bring all reagents to room temperature.

2. Add **50 µL** of **standard** or diluted (if necessary) **sample** to the appropriate wells.

3. Mix each reagent by inverting the reagent bottle prior to use.

4. Add **50 µL** of the **Histamine-HRP Conjugate** to each well. Use a multi-channel or repeater pipette if appropriate.

5. Mix by gently shaking the plate. A micro-plate shaker may be used if available.

6. Cover the plate with plastic film and incubate at room temperature (18-30°C) for 45 minutes.

7. Empty contents of wells into sink and blot on paper towel to remove as much fluid as possible.

8. Wash plate 3 times as follows:
   Add 300 µL **Working Wash Buffer** per well.
   Shake plate slightly during soak period for best wash results.
   Empty wash solution into sink by inversion then blot plate against clean paper towel to remove any remaining washing buffer.

9. Add 150 µL of substrate to each well. Be careful not to touch inner well walls. If possible use a multichannel pipette for best results. Mix by shaking plate gently.

10. Incubate at room temperature (18-30°C) for 30 minutes.

11. To ensure uniform color development, before recording absorbance gently shake the plate by sliding it back and forth on a flat surface or, if available, use the shaker function on the reader.

12. Measure the absorbance at 650 nm if not using Stop Solution else, add 75 uL Stop Solution (1N HCl)...Optional to each well then measure the absorbance at 450 nm.

Data Analysis:

1. A log-logit curve plot is recommended for generating a standard curve for this assay.
2. If a substrate blank was measured, subtract this value from all of the data before averaging replicate well absorbance values.
3. Calculate the percent of maximal binding (%B/B0) of each standard and sample by dividing the absorbance of the standard by the 0 standard absorbance and multiplying by 100. Transform this ratio into the logit function, wherein:

   logit = ln (%B/B0/(100-%B/B0)).

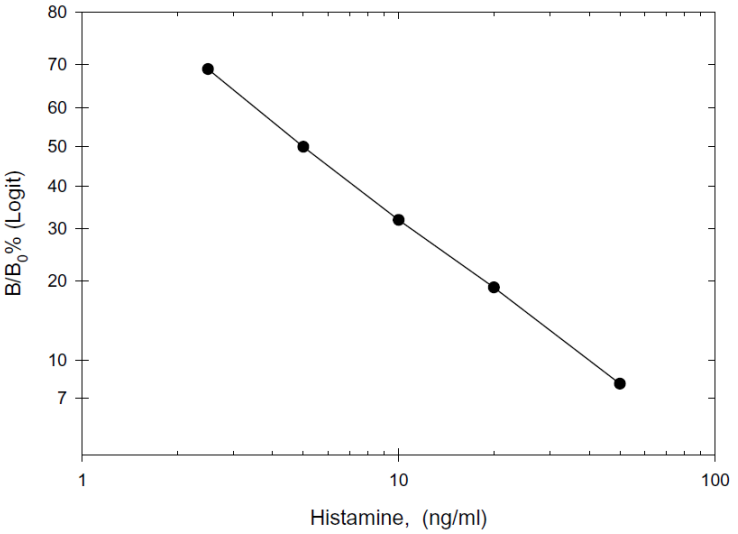
   Example: 0 Standard (Maximal) Absorbance = 1.800 = B0
   10 ng/mL Standard Absorbance = 1.000
   %B/B0 = 1.000/1.800 x 100 = 55.5
   Logit = ln(55.5/(100-55.5) = 0.221

4. Graph the standard curve by plotting the logit for each standard concentration on the Y-axis against the log of the standard concentrations on the X-axis. Depending on apparent curve shape fit the curve appropriately to obtain an equation for comparing samples to standard curve obtained.
6. Determine the %B/B0 and logit values for each sample:

   Example: 0 Standard Absorbance = 1.800 = B0
   10 ng/mL Standard Absorbance = 0.600
   %B/B0 = 0.600/1.800 x 100 = 33.3
   Logit = ln(33.3/(100-33.3) = -0.695.

7. Using the standard curve, the concentration of each sample can be determined by comparing the logit value of each sample to the corresponding concentration of Histamine standard.
8. If the samples were diluted, the concentration determined from the standard curve must be multiplied by the dilution factor. If the absorbance values of a sample fall outside the values of the 2.5 or 50 ng/mL standard, concentrate or dilute the sample as appropriate and retest.
9. To convert mass based concentration of Histamine into molarity the following equation can be used: ng/mL x 9.005 = nmole/L (nM).
   E.g. 1.0 ng/mL = 9 nM.

Data Analysis: (continued)
Example Standard Curve



Performance Details:

Sensitivity
Defined as the lowest concentration point on the calibration curve that this test can reliably detect Histamine. Sensitivity = 2.5 ng/mL

Effective Assay Range
2.5 - 50.0 ng/mL.

Precision
Intra-assay ≤ 10%
Inter-assay ≤ 10%

Specificity/Crossreactivity

Histamine	100.0%
Histidine	0.008%
Cadaverine	0.003%
Tyramine	<0.01%
Spermine	<0.01%
Putrescine	<0.01%
Trimethylamine	<0.01%

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Statement of Limited Warranty:

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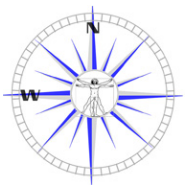
Notes:



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