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Histamine (HIS) ELISA Kit

rev 12/16

(Catalog # K4163-100, 100 assays, Store at 4°C)

I. Introduction:

Histamine is an organic nitrogenous compound involved in local immune responses as well as regulating physiological function in the gut and acting as a neurotransmitter. Histamine is involved in the inflammatory response and has a central role as a mediator of pruritus. As part of an immune response to foreign pathogens, histamine is produced by basophils and by mast cells found in nearby connective tissues. Histamine increases the permeability of the capillaries to white blood cells and some proteins, to allow them to engage pathogens in the infected tissues. This ELISA kit uses Competitive-ELISA as the method. The microtiter plate provided in this kit has been pre-coated with histamine. During the reaction, histamine in the sample or standard competes with a fixed amount of histamine on the solid phase supporter for sites on the Biotinylated Detection Antibody specific to histamine. The concentration of histamine in the samples is then determined by comparing the O.D. of the samples to the standard curve.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of Histamine.
Detection Range: 1.563 - 100 ng/ml
Sensitivity: < 0.938 ng/ml

III. Specificity:

Universal

IV. Sample Type:

Serum, plasma, tissue homogenates and other biological fluids

V. Kit Contents:

Components	K4163-100	Part No.
Micro ELISA Plate	8 X 12 strips	K4163-100-1
Lyophilized Standard	2 vials	K4163-100-2
Sample / Standard dilution buffer	20 ml	K4163-100-3
Biotin- detection antibody (Concentrated)	60 µl	K4163-100-4
Antibody dilution buffer	10 ml	K4163-100-5
HRP-Streptavidin Conjugate (SABC)	120 µl	K4163-100-6
SABC dilution buffer	10 ml	K4163-100-7
TMB substrate (Avoid light)	10 ml	K4163-100-8
Stop Solution	10 ml	K4163-100-9
Wash buffer (25X)	30 ml	K4163-100-10
Plate sealers	5	K4163-100-11

VI. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm
- 37°C incubator
- Precision pipettes with disposable tips
- Distilled or deionized water
- Clean eppendorf tubes for preparing standards or sample dilutions
- Absorbent paper

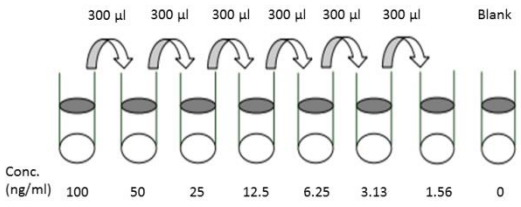
VII. Storage and Handling:

The entire kit may be stored at 4°C for up to 6 month from the date of shipment. Avoid freeze-thaw cycles.

VIII. Reagent Preparation:

Note: Prepare reagents within 30 minutes before the experiment.
Before using the kit, spin tubes and bring down all components to the bottom of tubes.

1. **Biotin-detection antibody working solution**: Calculate the total volume of the working solution: 0.05 ml / well × quantity of wells with additional 0.1 - 0.2 ml of the total volume. Dilute the Biotin- detection antibody with Antibody dilution buffer at 1:100 and mix thoroughly.
2. **HRP-Streptavidin Conjugate (SABC)**: Calculate the total volume of the working solution: 0.1 ml / well × quantity of wells with additional 0.1 - 0.2 ml of the total volume. Dilute the SABC with SABC dilution buffer at 1:100 and mix thoroughly.
3. **Wash Buffer**: Dilute 30 mL of Concentrated Wash Buffer into 750 mL of Wash Buffer with deionized or distilled water. Put unused solution back at 4°C. If crystals have formed in the concentrate, warm it with 40°C water bath and mix it gently until the crystals have completely dissolved. The solution should be cooled to room temperature before use.



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4. **Standard Preparation:**
   - Reconstitute the lyophilized Histamine standard by adding 1 ml of Standard/Sample Dilution Buffer to make the 100 ng/ml standard stock solution. Use within 2 hours after reconstituting.
   - Allow solution to sit at room temperature for 10 minutes, then gently vortex to mix completely.
   - Prepare 0.6 ml of 5 ng/ml top standard by adding 0.3 ml of the above stock solution in 0.3 ml of Standard/Sample Dilution Buffer. Perform 2-fold serial dilutions of the top standards to make the standard curve within the range of this assay.
   - Suggested standard points are: 100, 50 , 25, 12.5, 6.25, 3.13, 1.56, 0 ng/ml

5. Sample Preparation:

Note: Samples to be used within 5 days may be stored at 4°C, otherwise samples must be stored at -20°C (≤1 month) or -80°C (≤2 months) to avoid loss of bioactivity and contamination. Avoid multiple freeze-thaw cycles.

- **Serum**: Coagulate the serum for 2 hours at room temperature or overnight at 4°C. Centrifuge at approximately 1000xg for 20 min. Analyze the serum immediately or aliquot and store at -20°C.
- **Plasma**: Collect plasma with heparin or EDTA as the anticoagulant. Centrifuge for 15 min at 2-8°C at 1500 x g within 30 min of collection. For eliminating the platelet effect, suggesting that further centrifugation for 10 min at 2-8°C at 10000xg. Analyze immediately or aliquot and store frozen at -20°C.
- **Tissue homogenates**: Rinse the tissues with ice-cold PBS (0.01M, pH=7.4) to remove excess hemolysis blood thoroughly. Tissue pieces should be weighed and then minced to small pieces which will be homogenized in PBS (the volume depends on the weight of the tissue. 9 mL PBS would be appropriate for 1 g of tissue. Some protease inhibitor is recommended to add into the PBS.) with a glass homogenizer on ice. To further break the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifuged for 5 minutes at 5000×g to retrieve the supernatant.
- **Cell culture supernatant**: Centrifuge supernatant for 20 minutes to remove insoluble impurity and cell debris at 1000×g at 2 - 8°C. Collect the clear supernatant and carry out the assay immediately or aliquot and store at -20°C.
- **Other biological fluids:** Centrifuge samples for 20 min at 1000×g at 4°C. Collect the supernatant and carry out the assay immediately.
- End user should estimate the concentration of the target protein in the test sample first, and select a proper dilution factor to make the diluted target protein concentration falls the optimal detection range of the kit.

IX. Assay Protocol:

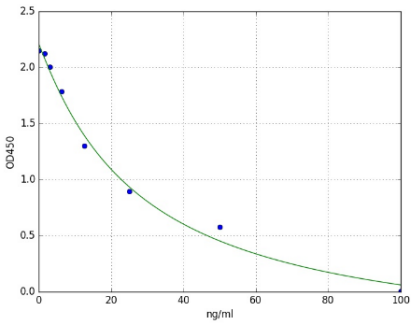
Note: Bring all reagents and samples to room temperature 30 minutes prior to the assay.
It is recommended that all standards and samples be run at least in duplicate.
A standard curve must be run with each assay.

1. Prepare all reagents, samples and standards as instructed in section VIII.
2. Wash plate 2 times with **1X Wash Solution** before adding standard, sample and control wells.
3. Add 50 µl of each **standard** and **samples** into appropriate wells. Immediately add 50 µL of **Biotinylated Detection Antibody working solution** to each well. Cover well and incubate for 45 minutes at 37ºC.
4. Discard the plate content and wash 3 times with **1X Wash Solution**. Wash by filling each well with Wash Buffer (350 µl) using a multi-channel pipette or auto-washer. Let it soak for 1-2 minutes, then remove all residual wash-liquid from the wells by aspiration. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Clap the plate on absorbent filter papers or other absorbent material.
5. Add 0.1 ml of **SABC working solution** into each well, cover the plate and incubate at 37ºC for 30 min.
6. Discard the solution and wash 5 times with **1X Wash Solution** as step 4.
7. Add 90 µl of **TMB substrate** into each well, cover the plate with a new plate sealer and incubate at 37 ºC in dark within 15-30 min. The shades of blue should be seen in the first 3-4 wells by the end of incubation.
8. Add 50 µl of **Stop Solution** to each well. The adding order of stop solution should be as the same as the substrate solution.
9. Read result at 450 nm within 20 minutes.

X. CALCULATION:

Average the duplicate readings for each standard and samples. The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The Histamine concentration of the samples can be interpolated from the standard curve. If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

Figure: Typical Standard Curve: These standard curves are for demonstration only. A standard curve must be run with each assay.



XI. RELATED PRODUCTS:

- Clemizole (Cat. No. 2481-10, -50)
- Histamine Assay Kit (Colorimetric) (Cat. No. K506-100)

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