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BioVision
BioVision Incorporated

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QuickDetect™ Hydroxylysine (Human) ELISA Kit

(Catalog # E4714-100 ; 96 assays ; Storage at 4ºC)

I. Introduction:

Hydroxylysine (Hyl) is an amino acid which arises from a post-translational hydroxy modification of lysine. It is most widely known as a component of collagen. QuickDetect™ Hydroxylysine (Human) ELISA Kit uses a double-antibody sandwich enzyme-linked immunosorbent one-step process assay to assay the level of hydroxylysine in samples. Standard, test sample and HRP-labeled hydroxylysine (antibodies were added to enzyme wells which are Pre-coated with hydroxylysine antibody, then carry out incubation and wash to remove the uncombined enzyme. Upon adding Chromogen Solution A and B, the color of the liquid will change into blue, and the reaction with the acid will cause the color to become yellow. The depth of color and the concentration of the hydroxylysine sample are positively correlated.

II. Applications:

- This ELISA kit is used for in vitro quantitative determination of Human Hydroxylysine
- Assay range: 9.3ng/ml- 300ng/ml
- Accuracy: Standard linear regression correlation coefficient R with the expected value of the concentration, greater than or equal to 0.9900.

III. Sample Type:

- Plasma
- Cell and tissue culture supernatants
- Serum
- Other biological fluids
- Tissue and cell lysates

IV. Kit Contents:

Components	E4714-100	Part Number
Micro ELISA Plate	8 X 12 strips	E4714-100-1
Standard（300 ng/ml）	0.6 ml	E4714-100-2
Standard diluent	6.0 ml	E4714-100-3
Special diluent	6.0 ml	E4714-100-4
HRP-Conjugate reagent	6.0 ml	E4714-100-5
Washing Buffer (20X)	25 ml	E4714-100-6
Chromogen Solution A	6.0 ml	E4714-100-7
Chromogen Solution B	6.0 ml	E4714-100-8
Stop Solution	6.0 ml	E4714-100-9
Microplate Sealers	2	E4714-100-10

V. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm

VI. Storage Conditions and Reagent Preparation:

The entire kit may be stored at 4°C for up to 6 months from the date of shipment.

1. Wash Buffer: Dilute 20X Wash Buffer to 1X with deionized or distilled water.

2. 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. Samples that contain NaN_3 cannot be used for this assay. Do not use heat-treated samples

- **Serum:** collect blood with non-pyrogenic and endotoxin tubes to avoid any cell stimulation. Centrifuge 3000 rpm for 10 minutes and separate the serum and red blood cells as quickly as possible. If precipitation appears, centrifuge again.
- **Plasma:** Collect plasma with heparin or EDTA as the anticoagulant. Mix for 20 minutes and centrifuge for 30 min at 2-8°C at 3000 rpm. For eliminating the platelet effect, suggesting that further centrifugation for 10 min at 2-8°C at 10000xg. Collect the supernatant and carry out the assay immediately. Avoid hemolysis, high cholesterol samples.
- **Cell culture supernatant:** Centrifuge supernatant for 20 minutes at 2000 – 3000 rpm to remove insoluble impurity and cell debris. Collect the clear supernatant and carry out the assay immediately or aliquot and store at -20°C.
- **Homogenate:** Homogenize with saline buffer and centrifuge for 10minutes at the speed of 3000 rpm, then get supernatant for detection.
- **Urine:** Collect with sterile container, centrifuge for 20 minutes at 2000-3000 rpm. Collect supernatant. If precipitation appears, centrifuge again. Use this description to also process hydrothorax and cerebrospinal fluid.
- **Tissue samples:** After cutting samples, check the weight, add PBS(PH7.4), Rapidly freeze with liquid nitrogen, keep samples at 2-8℃ after melting, add PBS（PH7.4）, Homogenize by hand or Grinders, centrifuge for 20 minutes at 2000-3000

rpm. Collect supernatant.

- 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 fall in the optimal detection range of the kit.
- **Storage:** Serum, plasma, and cell culture fluid samples should be used within 7 days, stored at 2-8℃, otherwise samples must store at -20℃ (2months) or -80℃ (6months) to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles. Take out the sample and wait until the temperature turns into room temperature before performing the assay.
- **DO NOT USE HEAT-TREATED SPECIMENS.**

3. Standard Preparation: Prepare 0.6 ml of 150 ng/ml standard by adding 0.3 ml of the 300 ng/ml stock solution in 0.3 ml of Standard Buffer. Perform 2-fold serial dilutions of the top standards to make the standard curve within the range of this assay. Use 0.3 ml standard diluent as blank control. • Suggested standard points are: 300, 150, 75, 37.5, 18.7, 0 ng/ml

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.

VII. 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 VI.

2. Set blank wells, standard wells, and test sample wells respectively:
(1) Blank well: do not add samples and horseradish peroxidase (HRP), other operations are the same.
(2) Standard wells: Add standard 50 µl to Standard wells.
(3) Test sample wells: Add 40µl of Special diluent and then add 10 µl of sample. (The final sample dilution is five times and the final result calculation should be multiplied by five times).
(4) Add 50µl of horseradish peroxidase (HRP) into each well, except blank well. Then seal the plate, and gently shake, then incubate 60 minutes at 37°C.

3. Discard the solution and wash 5 times with **1X Wash Solution.** Wash by filling each well with Wash Buffer (350 µl) using a multi-channel pipette or autowasher. Let it soak for 1-2 minutes, and 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 materials.

4. Add 50 µl of **Chromogen solution A** to each well, and then add 50 µl of **Chromogen solution B** to each well. Gently shake and incubate for 10 minutes at 37°C away from light.

5. Add **Stop Solution** 50 µl into each well to stop the reaction.

6. Measure the optical density (OD) at 450 nm wavelength within 15 minutes after adding the stop solution.

7. Calculation: Set blank well zero, measure the optical density (OD) at 450 nm. 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 Human Hydroxylysine 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.

VIII. Related Products:

Hydroxyproline Assay Kit (Perchlorate-Free) (K226)
Hydroxyproline Colorimetric Assay Kit (K555)
Hydroxyproline ELISA Kit (E4503)

FOR RESEARCH USE ONLY! Not to be used on humans.