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# QuickDetect™ 5-lipoxygenase (5-LOX) (Human) ELISA Kit

**( Catalog # E4704-100; 96 assays; Storage at 4°C )**

## I. Introduction:

BioVision's 5-lipoxygenase ELISA kit is based on Sandwich ELISA method. The Micro Elisa strip plate provided in this kit has been pre-coated with an antibody specific to 5-LOX. Standards or samples are added to the appropriate Micro Elisa strip plate wells and combined to the specific antibody. Then a Horseradish Peroxidase (HRP) conjugated antibody specific for 5-LOX is added to each Micro Elisa strip plate well and after incubation, all unbound components are washed away. The TMB substrate solution is added to each well. The wells that contain 5-LOX and HRP conjugated 5-LOX antibody will appear blue in color and turn yellow after the addition of the stop solution. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm. The OD value is proportional to the concentration of 5-LOX. The concentration of 5-LOX in the samples can be calculated by comparing the OD of the samples to the standard curve.

## II. Applications:

- Detection range: 1U/L - 480 U/L
- Assay Precision: Intra-Assay: CV<10%, Inter-Assay: CV<12%, CV (%) = SD/mean X 100
- Sensitivity: 1U/L

## III. Sample Type:

- Plasma
- Cell and tissue culture supernatants
- Serum
- Other biological fluids

## IV. Kit Contents:

| Components | E4704-100 | Part Number |
|---|---|---|
| Micro ELISA strip-plate | 1 | E4704-100-1 |
| Standard (960 U/L) | 0.5 ml | E4704-100-2 |
| Standard diluent | 6 ml | E4704-100-3 |
| HRP-Conjugate reagent | 10 ml | E4704-100-4 |
| Sample diluent | 6 ml | E4704-100-5 |
| Chromogen Solution B | 6 ml | E4704-100-6 |
| Chromogen Solution A | 6 ml | E4704-100-7 |
| Stop Solution | 6 ml | E4704-100-8 |
| Wash buffer (20X) | 25 ml | E4704-100-9 |
| Plate sealers | 2 | E4704-100-10 |

## V. User Supplied Reagents and Equipment:

- Plate reader capable of reading absorbance at 450 nm, Distilled or deionized water, 37°C incubator, absorbent paper

## VI. Storage Conditions and Reagent Preparation:

Store the kit at 2°C – 8°C in dark for up to 6 months from the date of shipment. Avoid freeze-thaw cycles.
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.
Wash Buffer: Dilute the concentrated washing buffer (20X) with distilled water.

## VII. Assay Protocol:

1. **Standard Preparation:** Ten wells are set for standards in a Micro Elisa strip plate. In Well 1 and Well 2, 50 µl Standard solution and 50 µl Standard Dilution buffer are added and mixed well. In Well 3 and Well 4, 50 µl solution from Well 1 and Well 2 are added respectively. Then 50 µl Standard Dilution buffer are added and mixed well. 50 µl solution is discarded from Well 3 and Well 4. In Well 5 and Well 6, 50 µl solution from Well 3 and Well 4 are added respectively. Then 50µl Standard Dilution buffer are added and mixed well. In Well 7 and Well 8, 50 µl solution from Well 5 and Well 6 are added respectively. Then 50µl Standard Dilution buffer are added and mixed well. In Well 9 and Well 10, 50µl solution from Well 7 and Well 8 are added respectively. Then 50µl Standard Dilution buffer are added and mixed well. 50 µl solution is discarded from Well 9 and Well 10. After dilution, the total volumes in all the wells are 50µl and the concentrations are 480 U/L, 240 U/L, 120 U/L, 60 U/L and 30 U/L, respectively.

2. **Sample preparation:**
   **Note:** Sample extraction and ELISA assay should be performed as soon as possible after sample collection. The samples should be extracted according to the relevant literature. If ELISA assay cannot be performed immediately, samples can be stored at -20°C. Repeated freeze-thaw cycles should be avoided. This ELISA kit cannot be used for samples with $NaN_3$ which

can inhibit the activity of HRP.

- **Serum samples:** After collection of the whole blood, allow the blood to clot by leaving it undisturbed at room temperature. This usually takes 10-20 minutes. Remove the clot by centrifuging at 2,000-3,000 rpm for 20 minutes. If precipitates appear during reservation, the sample should be centrifuged again.
- **Plasma samples:** Collect the whole blood into tubes with anticoagulant (EDTA or citrate). After incubated at room temperature for 10-20 minutes, tubes are centrifuged for 20 min at 2,000-3,000 rpm. Collect the supernatant carefully as plasma samples. If precipitates appear during reservation, the sample should be centrifuged again.
- **Urine samples:** Collect urine into aseptic tubes. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. If precipitates appear during reservation, the sample should be centrifuged again. The preparation procedure of cerebrospinal fluid and pleuroperitoneal fluid is the same as that of the urine sample.
- **Cell samples:** Collect culture supernatant into aseptic tubes. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. If you want to detect intracellular components, dilute the cells to 1X100/ml with PBS (pH 7.2-7.4). The cells were destroyed to release intracellular components by repeated freezing and thawing. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. If precipitates appear during reservation, the sample should be centrifuged again.
- **Tissue samples:** Tissue samples are cut, weighed, frozen in liquid nitrogen and stored at -80°C for future use. The tissue samples were homogenized after adding PBS (pH 7.4). Samples should be operated at 4°C. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. Aliquot the supernatant for ELISA assay and future use.
- **Note:** 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.

**3. 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. In the Micro Elisa strip plate, leave a well empty as blank control. In sample wells and 40 µl Sample dilution buffer and 10 µl sample are added (dilution factor is 5). Samples should be loaded onto the bottom without touching the well wall. Mix well with gentle shaking.
2. Add 100 µl HRP conjugate to each well except blank control. Cover the plate with plate membrane and incubate for 60 min at 37℃.
3. Remove plate sealer, aspirate the solution, air dry the plate and add washing buffer (1X). Discard the wash solution after incubating for 30 seconds. Repeat the washing procedure 5 times.
4. Add 50 µl Chromogen Solution A and 50 µl Chromogen Solution B to each well, mix with gently shaking and incubate at 37°C for 15 minutes in dark.
5. Add 50 µl Stop Solution to each well to stop the reaction. The blue color should change to yellow color.
6. Read absorbance at 450nm after Adding Stop Solution within 15 min after adding stop solution. The OD value of the blank control well is set as zero.

**4. Calculation:**

Known concentrations of Human 5-LOX Standard and its corresponding reading OD is plotted on the log scale (x-axis) and the log scale (y-axis) respectively. The concentration of Human 5-LOX in sample is determined by plotting the sample's O.D. on the Y-axis. The original concentration is calculated by multiplying the dilution factor.

## VIII. Related Products:

5-Lipoxygenase, Active, Human Recombinant (P1373)
Lipoxygenase Activity Assay Kit (Fluorometric) (K978)
5-Lipoxygenase Inhibitor Screening Kit (Fluorometric) (K980)

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