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# QuickDetect™ Alpha-enolase (ENO1) (Human) ELISA Kit

**( Catalog # E4706-100 ; 96 assay ; Storage at 4ºC )**

## I. Introduction:

BioVision's α-enolase 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 ENO1. 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 ENO1 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 ENO1 and HRP conjugated ENO1 antibody will appear blue in color and then 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 ENO1. The concentration of ENO1 in the samples can be calculated by comparing the OD of the samples to the standard curve.

## II. Applications:

- Assay Precision: Intra-Assay: CV<10%, Inter-Assay: CV<12%, CV (%) = SD/mean X 100
- Sensitivity: 0.1 ng/mL
- Detection range: 1.25 ng/mL - 40 ng/mL
- Detection of Alpha-enolase in human serum, plasma, culture media samples.

## III. Sample Type:

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

## IV. Kit Contents:

| Components | E4706-100 | Part Number |
|---|---|---|
| Micro Elisa strip plate | 1 | E4706-100-1 |
| Standard：80 ng/mL | 0.5 ml | E4706-100-2 |
| Standard diluent | 6 ml | E4706-100-3 |
| HRP-Conjugate reagent | 10 ml | E4706-100-4 |
| Sample diluent | 6 ml | E4706-100-5 |
| Chromogen Solution A | 6 ml | E4706-100-6 |
| Chromogen Solution B | 6 ml | E4706-100-7 |
| Stop Solution | 6 ml | E4706-100-8 |
| Wash solution (20X) | 25 ml | E4706-100-9 |
| Plate sealers | 2 | E4706-100-10 |

## V. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm
- Distilled or deionized water

## VI. Storage Conditions and Reagent Preparation:

The entire kit may be stored at 4°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.

1. **Wash Buffer:** Dilute the concentrated washing buffer (20X) with distilled water.

**2. Sample Preparation:** Note: Sample extraction and ELISA assay should be performed as soon as possible after sample collection. If ELISA assay cannot be performed immediately, samples can be stored at -20°C. Avoid multiple freeze-thaw cycles. Samples with $NaN_3$ should be avoided for this assay.
**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 urine sample.

**Cell samples:** If you want to detect the secretions of cells, 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.

**3. 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 50 µl 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 50 µl Standard Dilution buffer are added and mixed well. In Well 9 and Well 10, 50 µl solution from Well 7 and Well 8 are added respectively. Then 50 µl Standard Dilution buffer are added and mixed well. 50 µl solution is discarded from Well 9 and Well 10. After dilution, the total volume in all the wells are 50 µl and the concentrations are 40 ng/mL, 20 ng/mL, 10 ng/mL, 5 ng/mL and 2.5 ng/mL respectively.

## 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 VIII.
2. In sample wells, add 40 µl Sample dilution buffer and 10 µl samples are added (dilution factor is 5). Leave a well empty as blank control. Samples should be loaded onto the bottom without touching the well wall. Mix well with gentle shaking.
3. Add 100 µl HRP-Conjugate reagent to each well except the blank control well. Incubate 60 min at 37°C.
4. Remove plate sealer, aspirate and refill with the wash solution. Discard the wash solution after resting for 30 seconds. Repeat the washing procedure for 5 times
5. 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.
6. Add 50 µl stop solution to each well to terminate the reaction. The color in the well should change from blue to yellow.
7. Read absorbance O.D. at 450nm within 15 minutes after adding stop solution. The OD value of the blank control well is set as zero.

## VIII. Calculation:

Known concentrations of Human ENO1 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 ENO1 in the sample is determined by plotting the sample's O.D. on the Y-axis. The original concentration is calculated by multiplying the dilution factor.

## IX. Related Products:

Human Enolase α Inhibitor Screening Kit (Colorimetric) (K526)
Neuron Specific Enolase ELISA Kit (E4703)
Enolase Activity Colorimetric/Fluorometric Assay Kit (K691)
Human Recombinant Alpha-Enolase (6363)

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