



**ENO2 ELISA Kit (Human)
(OKCA00125)
Lot# KG0536**

Instruction for Use

For the quantitative measurement of Human ENO2 in serum, plasma, tissue homogenates.

Lot to lot variations can occur. Refer to the manual provided with the kit.

This product is intended for research use only.

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1. Background

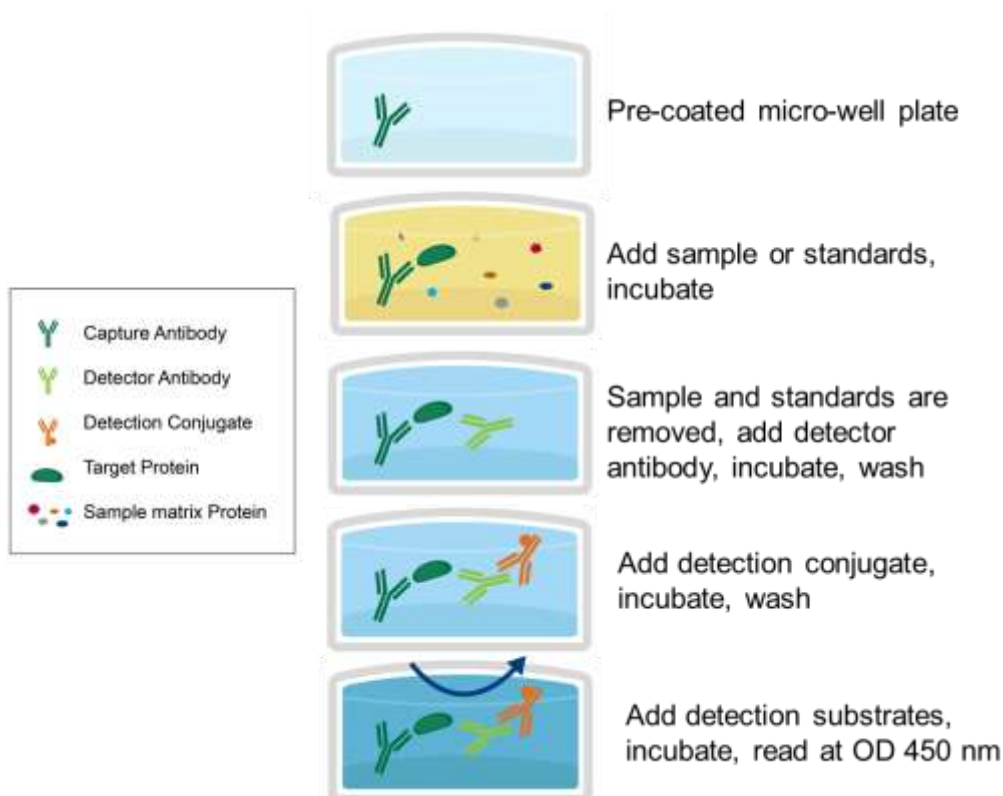
Principle

Aviva Systems Biology ENO2 ELISA Kit (Human) (OKCA00125) is based on standard sandwich enzyme-linked immuno-sorbent assay technology. A mouse monoclonal antibody specific for human ENO2 has been pre-coated onto a 96-well plate (12 x 8 Well Strips). Standards or test samples are added to the wells and incubated. After washing, a biotinylated detector antibody specific for human ENO2 is added, incubated and followed by washing. Avidin-Biotin-Peroxidase Complex is then added, incubated and unbound conjugate is washed away. An enzymatic reaction is produced through the addition of TMB substrate which is catalyzed by HRP to generating a blue color product that changes to yellow after adding acidic stop solution. The density of yellow coloration read by absorbance at 450 nm and is quantitatively proportional to the amount of sample human ENO2 captured in well.

General Specifications

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Range	0.78 – 50 ng/mL
LOD	< 0.195 ng/mL (Derived by linear regression of OD ₄₅₀ of the Mean Blank + 2xSD)
Specificity	Human ENO2 UniProt ID: P09104, Q96J33, A8K3B0 <u>GeneID</u> : 2026
Cross-Reactivity	No detectable cross-reactivity with other relevant proteins

2. Assay Summary



3. Storage and Stability

- Upon receipt store kit at 4°C. Do not use past expiration date.

4. Kit Components

- The following reagents are the provided contents of the kit.

Description	Quantity	Storage Conditions
Anti- ENO2 Microplate	96 Well (12 x 8 Well Strips)	Store at 4°C Do not use past expiration date
ENO2 Standard (Lyophilized)	2 x 50 ng	
100X Biotinylated Anti-ENO2Detector Antibody	1 x 120 µL	
100X HRP-Avidin	1 x 120 µL	
Detection Antibody Diluent	1 x 15 mL	
HRP-Avidin Diluent	1 x 15 mL	
Sample Diluent	1 x 50 mL	
25X Wash Buffer	1 x 20 mL	
TMB Substrate	1 x 10 mL	
Stop Solution	1 x 10 mL	

5. Precautions

- Read instructions fully prior to beginning use of the assay kit.
- Any deviations or modifications from the described method or use of other reagents could result in a reduction of performance.
- Reduce exposure to potentially harmful substances by wearing personal protective lab equipment including lab coats, gloves and glasses.
- For information on hazardous substances included in the kit please refer to the Material Safety Data Sheet (MSDS).
- Kit can not be used beyond the expiration date on the label.

6. Required Materials Not Supplied

- Microplate reader capable of reading absorbance at 450 nm.
- Automated plate washer (optional).
- Pipettes capable of precisely dispensing 0.5 μ L through 1 mL volumes of aqueous solutions.
- Pipettes or volumetric glassware capable of precisely measuring 1 mL through 100 mL of aqueous solutions.
- New, clean tubes and/or micro-centrifuge tubes for the preparation of standards or samples.
- Absorbent paper or paper toweling.
- Distilled or deionized ultrapure water.
- 37°C Incubator (optional).

7. Technical Application Tips

- Do not mix or substitute components from other kits.
- To ensure the validity of experimental operation, it is recommended that pilot experiments using standards and a small selection of sample dilutions to ensure optimal dilution range for quantitation.
- Samples exhibiting OD measurements higher than the highest standard should be diluted further in the appropriate sample dilution buffers.
- Prior to using the kit, briefly spin component tubes to collect all reagents at the bottom.
- Replicate wells are recommended for standards and samples.
- Cover microplate while incubating to prevent evaporation.
- Do not allow the microplate wells dry at any point during the assay procedure.
- Do not reuse tips or tube to prevent cross contamination.
- Avoid causing bubbles or foaming when pipetting, mixing or reconstituting.
- Completely remove of all liquids when washing to prevent cross contamination.
- Prepare reagents immediately prior to use and do not store, with the exception of the top standard.
- Equilibrate all materials to ambient room temperature prior to use (standards exception).
- For optimal results for inter- and intra-assay consistency, equilibrate all materials to room temperature prior to performing assay (standards exception) and perform all incubations at 37°C.
- Pipetting less than 1 μ L is not recommended for optimal assay accuracy.
- Once the procedure has been started, all steps should be completed without interruption. Ensure that all reagents, materials and devices are ready at the appropriate time.
- Incubation times will affect results. All wells should be handled in the same sequential order and time intervals for optimal results.
- Samples containing precipitates or fibrin strands or which are hemolytic or lipemic might cause inaccurate results due to interfering factors.
- TMB Substrate is easily contaminated and should be colorless or light blue until added to plate. Handle carefully and protect from light.

8. Reagent Preparation

- Equilibrate all materials to room temperature prior to use and use immediately.

8.1 Human ENO2 Assay Standards

- 8.1.1 Prepare the human ENO2 **standards** no greater than 2 hours prior to performing experiment. Standards should be held on ice until use in the experiment.
- 8.1.2 Reconstitute one vial of the provided 50 ng **Lyophilized ENO2 Standard** for each experiment. Prepare the stock 50 ng/mL **ENO2 Standard** by reconstituting one tube of **Lyophilized ENO2 Standard** as follows:
 - 8.1.2.1 Gently spin or tap the vial at 6,000 – 10,000 rpm for 30 seconds to collect all material at the bottom.
 - 8.1.2.2 Add 1 mL of **Sample Diluent** to the vial.
 - 8.1.2.3 Seal the vial then mix gently and thoroughly.
 - 8.1.2.4 Leave the vial at ambient temperature for 15 minutes.
- 8.1.3 Prepare a set of seven serially diluted standards as follows:
 - 8.1.3.1 Label tubes with numbers 2 – 8.
 - 8.1.3.2 Use the undiluted 50 ng/mL ENO2 **Standard** as the high standard point (Tube #1).
 - 8.1.3.3 Add 300 µL of **Sample Diluent** to Tube #'s 2 – 8.
 - 8.1.3.4 Prepare **Standard #2** by adding 300 µL of 50 ng/mL ENO2 (Tube #1) to Tube #2. Mix gently and thoroughly.
 - 8.1.3.5 Prepare **Standard #3** by adding 300 µL of **Standard #2** from Tube #2 to Tube #3. Mix gently and thoroughly.
 - 8.1.3.6 Prepare further serial dilutions through Tube #7. Reference the table below as a guide for serial dilution scheme.
 - 8.1.3.7 Tube #8 is a blank standard (only **Sample Diluent**), which should be included with every experiment.

Standard Number (Tube)	Standard To Dilute	Volume Standard to Dilute (µL)	Volume Sample Diluent (µL)	Total Volume (µL)	Final Concentration
1	50 ng Lyophilized ENO2 Standard	NA	1,000	1,000	50 ng/mL
2	50 ng/mL	300	300	600	25 ng/mL
3	25 ng/mL	300	300	600	12.5 ng/mL
4	12.5 ng/mL	300	300	600	6.25 ng/mL
5	6.25 ng/mL	300	300	600	3.12 ng/mL
6	3.12 ng/mL	300	300	600	1.56 ng/mL
7	1.56 ng/mL	300	300	600	0.78 ng/mL
8	NA	0	300	300	0.0 (Blank)



8.2 Microplate Preparation

- Micro-plates are provided ready to use and do not require rinsing or blocking.
- Unused well strips should be returned to the original packaging, sealed and stored at 4°C.
- Equilibrate microplates to ambient temperatures prior to opening to reduce potential condensation.

8.3 1X Wash Buffer

- 8.3.1 If crystals have formed in the **25X Wash Buffer** concentrate, equilibrate to room temperature and mix gently until crystals have completely dissolved.
- 8.3.2 Add the entire 20 mL contents of the **25X Wash Buffer** bottle to 480 mL of ultra-pure water to a clean > 500 mL bottle or other vessel.
- 8.3.3 Seal and mix gently by inversion. Avoid foaming or bubbles.
- 8.3.4 Store the **1X Wash Buffer** at room temperature until ready to use in the procedure. Store the prepared **1X Wash Buffer** at 4°C for no longer than 1 week. Do not freeze.

8.4 1X Biotinylated Anti-ENO2 Detector Antibody

- 8.4.1 Prepare the **1X Biotinylated Anti-ENO2 Detector Antibody** immediately prior to use by diluting the **100X Biotinylated Anti- ENO2 Detector Antibody** 1:100 with **Antibody Diluent Buffer**.
- 8.4.2 For each well strip to be used in the experiment (8-wells) prepare 1,000 µL by adding 10 µL of **100X Biotinylated Anti-ENO2 Detector Antibody** to 990 µL **Antibody Diluent Buffer**.
- 8.4.3 Mix thoroughly and gently. Hold no longer than 2 hours prior to using in procedure.

8.5 1X HRP-Avidin

- 8.5.1 Prepare the **1X HRP-Avidin** immediately prior to use by diluting the **100X HRP-Avidin** 1:100 with **HRP-Avidin Diluent**.
- 8.5.2 For each well strip to be used in the experiment (8-wells) prepare 1,000 µL by adding 10 µL of **100X HRP-Avidin** to 990 µL **HRP-Avidin Diluent**.
- 8.5.3 Mix thoroughly and gently. Hold no longer than 2 hours prior to using in procedure.

9. Sample Preparation

9.1 Sample Preparation and Storage

- Store samples to be assayed at 2-8°C for 24 hours prior being assayed.
- For long term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.
- Samples not indicated in the manual must be tested to determine if the kit is valid.
- Prepare samples as follows:
 - **Serum** - Use serum separator tube (SST) and allow samples to clot for two hours at room temperature or overnight at 4°C before centrifugation for 15 minutes at 1,000 x g. Remove serum and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.
 - **Plasma** - Collect plasma using EDTA, or heparin as an anticoagulant. Centrifuge for 15 minutes at 1,000 x g at 2-8°C within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.
 - **Tissue Homogenates** – 100 mg tissue was rinsed with 1X PBS, homogenized in 1 ml of 1X PBS and stored overnight at -20°C. After two freeze-thaw cycles were performed to break the cell membranes, the homogenates were centrifuged for 5 minutes at 5,000 x g, 2-8°C. The supernatant was removed and assayed immediately. Alternatively, aliquot and store samples at -20°C or -80°C. Centrifuge the sample again after thawing before the assay. Avoid repeated freeze-thaw cycles.

10. Assay Procedure

- Equilibrate all reagents and materials to ambient room temperature prior to use in the procedure.
- Optionally, to control for small potential variations in micro well-plate and day to day ambient temperature fluctuations, equilibrate all reagents prior to use and perform all incubation steps at to 37°C for optimal consistency and reproducibility.

- 10.1** Determine the required number of wells and return any remaining unused wells and desiccant to the pouch.
- 10.2** Add 100 µL of serially titrated standards, diluted samples or blank into wells of the ENO2 Microplate. At least two replicates of each standard, sample or blank is recommended.
- 10.3** Cover the plate with the plate sealer and incubate for 2 hours.
- 10.4** Remove the plate sealer and discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.
- 10.5** Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.
- 10.6** Add 100 µL of prepared **1X Biotinylated Anti-ENO2 Antibody** to each well.
- 10.7** Cover with the plate sealer and incubate for 60 minutes at 37°C.
- 10.8** Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.
- 10.9** Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.
- 10.10** Wash plate 3 times with **1X Wash Buffer** as follows:
 - 10.10.1 Add 200 µL of **1X Wash Buffer** to each assay well.
 - 10.10.2 Incubate for 1 minute.
 - 10.10.3 Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle.
 - 10.10.4 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.
 - 10.10.5 Repeat steps 10.10.1 through 10.10.4 **two** more times.
- 10.11** Add 100 µL of prepared **1X HRP-Avidin** into each well and incubate for 60 minutes at 37°C.
- 10.12** Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.
- 10.13** Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.
- 10.14** Wash plate **5 times** with **1X Wash Buffer** as in Step 10.10.
- 10.15** Add 90 µL of **TMB Substrate** to each well, cover with plate sealer and incubate **in the dark** for 15-30 minutes. Wells should change to gradations of blue. If the color is too deep, reduce the incubation time.
(NOTE: optimal incubation time must be determined by the user. Optimal development can be visualized by blue shading in the top four standard wells, while the remaining standards are still clear.)
- 10.16** Add 50 µL of **Stop Solution** to each well. Well color should change to yellow immediately. Add the **Stop Solution** in the same well order as done for the **TMB Substrate**.
- 10.17** Read the O.D. absorbance at 450 nm with a standard microplate reader within 5 minutes of stopping the reaction in step 10.16. If wavelength correction is available, set to 540 nm or 570 nm.

11. Calculation of Results

For analysis of the assay results, calculate the **Relative OD₄₅₀** for each test or standard well as follows:

$$(\text{Relative OD}_{450}) = (\text{Well OD}_{450}) - (\text{Mean Blank Well OD}_{450})$$

The standard curve is generated by plotting the mean replicate **Relative OD₄₅₀** of each standard serial dilution point vs. the respective standard concentration. The human ENO2 concentration contained in the samples can be interpolated by using linear regression of each mean sample **Relative OD₄₅₀** against the standard curve. This is best achieved using curve fitting software.

Note: if wavelength correction readings were available, subtract the readings at 540 nm or 570 nm from the readings at 450 nm. This may provide greater reading accuracy.

Note: if the samples measured were diluted, multiply the derived mean sample concentration by the dilution factor for a final sample concentration.

12. Typical Expected Data

12.1 Reproducibility

Intra-assay Precision: 3 samples with known low, middle and high levels ENO2 were tested with 20 replicates on one plate, respectively.

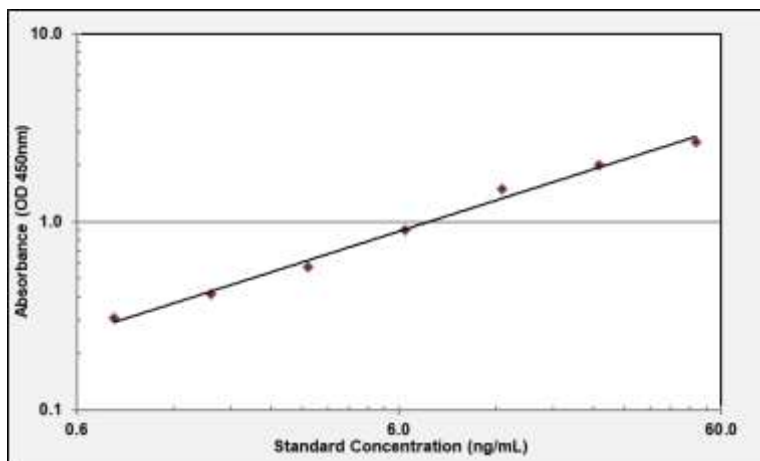
Inter-assay Precision: 3 samples with known low, middle and high level ENO2 were tested on 3 different plates, 8 replicates in each plate.

Mean Intra-Assay: CV \leq 8%

Mean Inter-Assay: CV \leq 10%

12.2 Typical standard curve

This standard curve is for demonstration purposes only. An assay specific standard curve should be performed with each assay.



Standard ng/mL	Absorbance (OD 450nm)
50	2.653
25	2.010
12.5	1.492
6.25	0.901
3.13	0.574
1.56	0.411
0.78	0.306

13. Technical Resources

Technical Support:

For optimal service please be prepared to supply the lot number of the kit used.

USA

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