Endothelin 1 ELISA Kit (Fluorometric)

Endothelin 1 ELISA Kit (Fluorometric) is an Enzyme Immunoassay kit for the quantification of Endothelin 1 in plasma.

Catalog number: ARG82248

Package: 96 wells

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INTRODUCTION

Endothelin 1 (ET-1), also known as preproendothelin-1 (PPET1), is a potent vasoconstrictor that in humans is encoded by the EDN1 gene and produced by vascular endothelial cells. The protein encoded by this gene is proteolytically processed to release a secreted peptide termed endothelin 1. Endothelin 1 is one of three isoforms of human endothelin. [Provided by Wikipedia: Endothelin 1]

PRINCIPLE OF THE ASSAY

This assay employs the competitive quantitative enzyme immunoassay technique. A secondary antibody that can bind to the Fc fragment of the primary antibody has been pre-coated onto a microplate. Add Primary Antibody to reaction wells and incubation. After wash step, peptide containing samples or standards and a Biotinylated-peptide are given into the wells of the microtiter plate. Biotinylated-peptide and free peptide compete for the antibody binding sites. After incubation, the wells are washed with Wash Buffer to remove unbound material. Then add Streptavidin-HRP to the reaction wells. After wash step, Substrate is added to the wells and fluorescence intensity in inversely proportion to the amount of free Endothelin 1 peptide present in the samples. Add Stop Solution to stop the reaction and read with a standard 96-well fluorometric plate reader (Ex. 315-340 nm/Em. 370-470 nm).

MATERIALS PROVIDED & STORAGE INFORMATION

Store all components at 4°C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody Coated Microplate	8 X 12 strips	4°C
Primary Antibody	1 vial	4°C
Standard Peptide	1 vial	4°C
Biotinylated-Peptide	1 vial	4°C
Streptavidin-HRP Conjugate	30 µL	4°C
20X Assay Buffer	50 mL	4°C
Positive Control	1 vial	4°C
Stable Peroxide Solution	1.5 mL	4°C
Substrate	12 mL (ready to use)	4°C (protect from light)
STOP Solution	12 mL (ready to use)	4°C
Acetate Plate Sealer (APS)	3 ea	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Fluorescence microplate reader capable of reading excitation at 315-340 nm and emission at 370-470 nm
- Deionized or distilled water
- Microplate shaker

- Pipettes and pipette tips
- Aprotinin (0.6TIU/mL of blood) (optional) Cat# RK-APRO
- Buffer A (optional) Cat#RK-BA-1
- Buffer B (optional) Cat#RK-BB-1
- C18 SEP-COLUMN (optional) Cat#RK-SEPCOL-1
- Microplate shaker of 300-400 rpm (recommended)
- Microtiter plate washer (recommended)

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Prior to beginning the assay procedure, bring all reagents and required number of strips to room temperature (20-23°C).
- Remove the number of strips required and return unused strips to the pack and reseal.
- Avoid air bubbles in the wells as this could result in lower binding efficiency and higher CV% of duplicate reading.
- Briefly spin down the all vials before use.
- If crystals are observed in the 20X Assay Buffer, warm to 37°C until the crystals are completely dissolved.
- Minimize lag time between wash steps to ensure the plate does not become completely dry during the assay.
- Ensure complete reconstitution and dilution of reagents prior to use.
- Take care not to contaminate the TMB Substrate. Do not expose the TMB

solution to glass, foil or metal. Do NOT return leftover TMB Substrate to bottle. Do NOT contaminate the unused TMB Substrate. If the solution is blue before use, DO NOT USE IT.

- Change pipette tips between the addition of different reagent or samples.
- Taping the well strips together with lab tape can be done as an extra precaution to avoid plate strips from coming loose during the procedure.
- Run both standards and samples in at least duplicates.

SAMPLE COLLECTION & STORAGE INFORMATION

The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design.

Plasma: Collect blood with EDTA, heparin or citrate tube and gently mix. Transfer the blood from the tubes to the centrifuge tubes containing aprotinin (0.6TIU/ml of blood) and gently mix for several times to inhibit the activity of proteinases. Centrifuge the blood at 1,600 x g for 15 minutes at 4°C and collect the plasma.

Note:

1. Do not use haemolytic, icteric or lipaemic specimens.
2. After collection, sample should be assayed immediately or store at ≤-70°C for 1 month. Avoid repeated freeze and thaw.

Extraction of peptide from plasma:

1. Acidify the plasma with an equal amount of buffer A (E.g., mix 1 mL of plasma with 1 mL of buffer A). Centrifuge at 6,000 to 17,000 x g for 20 minutes at 4°C.

2. Equilibrate a SEP-COLUMN containing 200 mg of C18 by washing with buffer B (1 mL, once) followed by buffer A (3 mL, 3 times)

Note: From step 3-5, no pressure should be applied to the column.

3. Load the acidified plasma solution onto the pre-equilibrated C-18 SEP-Column.
4. Slowly wash the column with buffer A (3 mL, twice) and discard the wash.
5. Elute the peptide slowly with buffer B (3 mL, once) and collect the eluent into a polystyrene tube.
6. Evaporate eluent to dryness in a centrifugal concentrator or by a suitable substitute method.
7. Keep the dried extract at -20°C and perform the assay as soon as possible. Use 1X Assay Buffer to reconstitute the dried extract. If the prptide value does not fall within the range of detection, dilute or concentration the sample accordingly.

REAGENT PREPARATION

- 1X Assay Buffer: dilute 20X Assay Buffer into distilled water to yield 1X Wash Buffer (E.g., add 50 mL of 20X Assay Buffer into 950 mL of distilled water to a final volume of 1000 mL).
- Primary Antibody: rehydrate the Primary Antibody with 1X Assay Buffer. Refer to QC datasheet for instruction on rehydrating the Primary Antibody. Allow to sit for at least 5 minutes to completely dissolve. Mix thoroughly.
- Positive Control: rehydrate the Positive Control with 1X Assay Buffer. Refer to QC datasheet for instruction on rehydrating the Positive Control. Allow

to sit for at least 5 minutes to completely dissolve. Mix thoroughly.

- **Biotinylated Peptide:** rehydrate the Biotinylated Peptide with 1X Assay Buffer. Refer to QC datasheet for instruction on rehydrating the Biotinylated Peptide. Allow to sit for at least 5 minutes to completely dissolve. Mix thoroughly.
- **Working Streptavidin-HRP Conjugate:** centrifuge the Streptavidin-HRP Conjugate vial provided in this kit (3,000-5,000 rpm, 5 seconds). Add 12 µL of Streptavidin-HRP Conjugate into 12 mL of 1X Assay Buffer to make Working Streptavidin-HRP Conjugate, vortex thoroughly.
- **Standards:** the Standards is reconstituted with 1 mL of 1X Assay Buffer. Gently vortex to mix well. The reconstituted Standard stock conc. is 1000 ng/mL. Allow the solution to sit at least 10 minutes at room temperature to completely dissolve in solution. Dilute each Standard as follow;

Standard tube	Endothelin 1 conc.	1X Assay Buffer (µL)	Standard stock, 1000 ng/mL (µL)
Stock	1000 ng/mL	0	1000
S1	100 ng/mL	900	100 of S1
S2	10000 pg/mL	900	100 of S2
S3	1000 pg/mL	900	100 of S3
S4	100 pg/mL	900	100 of S4
S5	10 pg/mL	900	100 of S5
S6	1 pg/mL	900	100 of S6

Note: Diluted Standards for serum / plasma with 1X Assay Buffer and for cell culture supernatant with medium.

ASSAY PROCEDURE

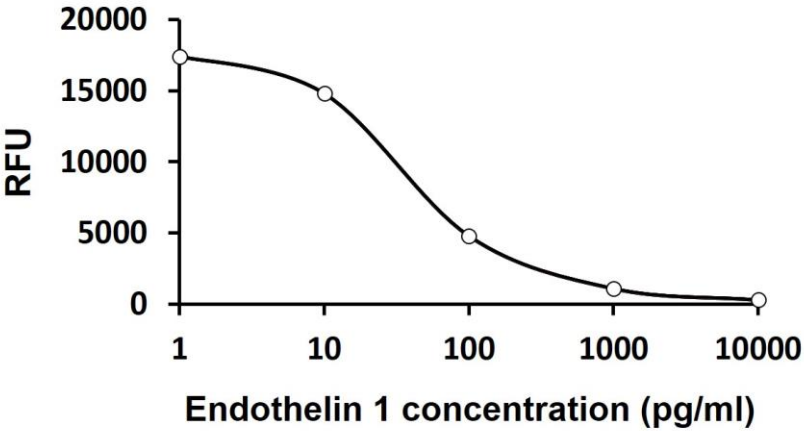
All materials should be equilibrated to room temperature (RT, 20-23°C) before use. Standards, control and samples should be assayed in duplicates.

1. Leave wells **A1** and **A2** empty as **Blank**.
2. Add **50 µL** of **1X Assay Buffer** into **B1** and **B2** well as **Total Binding.**
3. Add **50 µL** of **each diluted Standard (S6 to S2)** into wells from **C-1 and C-2** to **G-1 and G-2** respectively.
4. Add **50 µL** of **Positive Control** into wells **H-1** and **H-2**.
5. Add **50 µL** of **prepared samples** into the appropriate wells**.**
6. Add **25 µL** of **Primary Antibody** into each well **except the Blank well**.
7. Seal the plate with acetate plate sealer (APS). Incubate the plate **overnight** (**approximately 16-24 hours**) at **4°C**.
8. Next day, remove APS from plate. **DO NOT WASH THE PLATE.** Add **25 µL** of **Biotinylated Peptide** into each well **except the Blank well**.
9. Seal the plate with acetate plate sealer (APS). Incubate the plate for **1.5 hours** at **room temperature** on a microplate shaker (**300-400 rpm** is recommended).
10. Remove APS from plate. Discard content of the wells.
11. Aspirate each well and wash, repeating the process 3 times for a total **4 washes.** Wash by filling each well with **1× Assay Buffer (350 µL)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.

12. Add 100 µL of Working Streptavidin-HRP Conjugate into each well.
13. Reseal the plate with acetate plate sealer (APS). Incubate the plate for 1 hour at room temperature on a microplate shaker (300-400 rpm is recommended).
14. Mix 9 parts of Substrate to 1 part of Stable Peroxide Solution. This working solution is stable for 24 hours at room temperature and no protection from light is required. To reduce variability, equilibrate the working solution to room temperature before adding to the wells.
15. Remove APS from plate.
16. Aspirate and wash each well as step 11.
17. Add 100 µL of Working Substrate Solution to each well. Gently mix well.
18. Reseal the plate with acetate plate sealer (APS). Incubate the plate for 15-20 minutes at room temperature on a microplate shaker (300-400 rpm is recommended).
19. Remove APS from plate. Add 100 µL of Stop Solution into each well. Gently tap the plate to ensure thorough mixing. Proceed to the next step within 20 minutes.
20. Load the microplate onto a fluorescence microplate reader, and measure relative fluorescence units (RFU) of each well. The excitation and emission maxima for the Substrate Solution are 325 and 420 nm, respectively. Wavelengths between 315 and 340 nm for excitation and 370 and 470 nm for emission also can be used for detection.

EXAMPLE OF TYPICAL STANDARD VALUES

The following figures demonstrate typical results with the Endothelin 1 ELISA Kit (Fluorometric). One should use the data below for reference only. This data should not be used to interpret actual results.



CALCULATION OF RESULTS

1. Calculate the average RFU values for each set of standards and samples.
2. Construct a standard curve by plotting the known concentrations of standard peptide on the log scale (X-axis), and its corresponding fluorescence intensity reading on the linear scale (Y-axis).
3. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give

slightly different results.

4. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)
5. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

QUALITY ASSURANCE

Sensitivity

17.8 pg/mL