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Human Endothelin 1/ET-1 Competitive ELISA Kit

Catalog Number: EK1314
Size: 48 Test, 96 Test, 2 × 96 Test, 5 × 96 Test, 10 × 96 Test

For the quantitative determination of human Endothelin 1 (ET-1) concentration in cell culture supernates, serum and plasma.

This package insert must be read entirely before using this product. For proper performance, follow the protocol provided with each individual kit.

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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ASSAY PROCEDURE SUMMARY

1. **Prepare all reagents and standards as directed.**

2. **Add 50 µL of diluted *Antibody* to each well except Blank, NSB and TA wells.**

3. **Incubate for 1.5 hours at 22-28℃. Aspirate and wash 6 times.**

4. **Add 100 µL of serial diluted *Standard* to Standard wells. Add 100 µL of prepared samples to Sample wells. Add 100 µL of *Standard Diluent* or cell culture medium to NSB and B_0 wells. (The treatment refers to the Sample Preparation on Page 6).**

5. **Add 50 µL of diluted *ET-1 Conjugate* to each well except Blank and TA wells.**

6. **Incubate for 2 hours at 22-28℃. Aspirate and wash 6 times.**

7. **Add 5 µL of diluted *ET-1 Conjugate* to TA well.**

8. **Add 100 µL *Substrate Solution* to each well. Incubate for 5 - 30 minutes at 22-28℃. Protect from light.**

9. **Add 100 µL *Stop Solution* to each well.**

10. **Read at 450 nm within 30 minutes. Correction 570 or 630 nm.**

DESCRIPTION

Endothelin-1 (ET-1) is a polypeptide composed of 21 amino acid residues, a pleiotropic component, which was originally a 212 amino acid pre-polypeptide-propeptide. Vascular endothelial cells are a rich source of ET-1. The expression of ET-1 in endothelial cells is influenced by a variety of factors, including mechanical stimulation, multiple hormones, and pro-inflammatory cytokines. Endothelin was first identified as an endothelin-derived diastolic factor and then as an endothelin-derived contractile factor. ET-1 stimulates myocardial contraction and cardiomyocyte growth, regulates the release of vasoactive substances, and stimulates smooth muscle cell mitosis. It also acts as a growth promoting factor for endothelial cells, and its secretion is regulated by hypothalamic and pituitary cells. ET-1 may control the inflammatory response by promoting the adhesion and migration of neutrophils and stimulating the production of pro-inflammatory cytokines

PRINCIPLE OF THE ASSAY

This assay employs a solid-phase competitive Enzyme-Linked Immunosorbent Assay (ELISA) technique. A monoclonal antibody specific for human CNTF has been pre-coated onto a microplate. The biotin-linked ET-1 and purified ET-1/sample is added into the wells, and is bound by the immobilized antibody following incubation. After washing away any unbound substances, streptavidin-HRP (SA-HRP) is added. After washing, substrate solution is added to the wells and color develops in inverse proportion to the amount of ET-1. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- Do not use expired kit or reagents.
- Do not use reagents from other lots or manufacturers. Do not prepare component by yourself.
- If concentration of assayed factor in samples is higher than the highest standard, dilute the serum/plasma samples with *Assay Buffer*, dilute the cell culture supernate samples with *cell culture medium*. Reanalyze these and multiply results by the appropriate dilution factor.
- Any variation in testing personnel, sample preparation, standard dilution, pipetting technique, washing techniques, incubation time, temperature, kit age and equipment can cause variation in results.
- This assay is designed to eliminate interference by factors present in biological samples. Until all factors have been tested in the ELISA immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED (96 Test)

Unopened kit should be stored at 2 - 8℃.

- **ET-1 Antibody Microplate** (1 plate): 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against human ET-1.
- **ET-1 Standard** (2 vials): Recombinant ET-1 in a buffered protein base with preservatives; lyophilized.
- **ET-1 Conjugate** (1 vial, 70 µL): Recombinant HRP-conjugated ET-1 in a stabilizing solution; 100× liquid.
- **Standard Diluent** (1 bottle, 5 mL): In some, very rare cases, an insoluble precipitate of stabilizing protein has been seen in the Standard Diluent. This precipitate does not interfere in any way with the performance of the test and can thus be ignored.
- **Streptavidin-HRP** (1 vial, 140 µL): 100× liquid.
- **Assay Buffer (10×)** (1 bottle, 5 mL): PBS with 0.5 % Tween-20 and 5 % BSA.
- **Substrate** (1 bottle, 11 mL): TMB (tetramethyl-benzidine).
- **Stop Solution** (1 bottle, 11 mL): 0.18 M sulfuric acid.
- **Washing Buffer (20×)** (1 bottle, 50 mL): PBS with 1 % Tween-20.
- **Plate Covers** (6 strips).

STORAGE

Store kit reagents between 2 and 8 ℃. Immediately after use remaining reagents should be returned to cold storage (2 to 8℃). Expiry of the kit and reagents is stated on labels.
Expiration date of the kit components can only be guaranteed if the components are stored properly, and if, in case of repeated use of one component, this reagent is not contaminated by the first handling.

Unopened kit		Store at 2 - 8℃ (See expiration date on the label).
Opened/ Reconstituted Reagents	1× Washing Buffer 1× Assay Buffer Stop Solution Standard Diluent Substrate TMB ET-1 Conjugate Streptavidin-HRP	Up to 1 month at 2 - 8℃.
	ET-1 Standard	Up to 1 month at ≤ -20 ℃ in a manual defrost freezer. Discard after use.
	Microplate Wells	Up to 1 month at 2 - 8℃. Return unused strips to the foil pouch containing the desiccant pack, reseal along entire edge to maintain plate integrity.

Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- **Microplate reader** capable of measuring absorbance at 450 nm, with correction wavelength set at 570 nm or 630 nm.
- **Pipettes and pipette tips**.
- 50 µL to 300 µL adjustable **multichannel micropipette** with disposable tips.
- Multichannel micropipette **reservoir**.
- **Beakers, flasks, cylinders** necessary for preparation of reagents.
- **Deionized or distilled water**.
- **Polypropylene** test tubes for dilution.

PRECAUTION

- All chemicals should be considered as potentially hazardous.
- We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves.
- Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statement(s) for specific advice.
- The Stop Solution provided with this kit is an acid solution. Wear eyes, hand, face, and clothing protection when using this material.
- Reagents are intended for research use only and are not for use in diagnostic or therapeutic procedures.
- Do not mix or substitute reagents with those from other lots or other sources.
- Do not use kit reagents beyond expiration date on label.
- Do not expose kit reagents to strong light during storage and incubation.
- Do not eat or smoke in areas where kit reagents or samples are handled.
- Avoid contact of skin or mucous membranes with kit reagents or specimens.
- Rubber or disposable latex gloves should be worn while handling kit reagents or specimens.
- Avoid contact of substrate solution with oxidizing agents and metal.
- Avoid splashing or generation of aerosols.
- In order to avoid microbial contamination or cross- contamination of reagents or specimens which may invalidate the test use disposable pipette tips and/or pipettes.
- Use clean, dedicated reagent trays for dispensing the conjugate and substrate reagent.
- Exposure to acid inactivates the HRP and antibody conjugate.
- Glass-distilled water or deionized water must be used for reagent preparation.
- Substrate solution must be warmed to room temperature prior to use.
- Decontaminate and dispose specimens and all potentially contaminated materials as they could contain infectious agents. The preferred method of decontamination is autoclaving for a minimum of 1 hour at 121.5℃.
- Liquid wastes not containing acid and neutralized waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 1.0 % sodium hypochlorite. Allow 30 minutes for effective decontamination. Liquid waste containing acid must be neutralized prior to the addition of sodium hypochlorite.
- In some cases, an insoluble precipitate of stabilizing protein has been seen in the Standard Diluent. This precipitate does not interfere in any way with the performance of the test and can thus be ignored. Or remove precipitate by centrifuging at 6,000 × g for 5 minutes.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- When using an automated plate washer, adding a 30 seconds soak period before washing step and/or rotating the plate between wash steps may improve assay precision.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution.
- The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.
- It is recommended that all samples and standards be assayed in duplicate.
- Take care not to scratch the inner surface of the microwells.

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates – Remove particulates by centrifugation at 300 × g for 10 minutes and assay immediately or aliquot and store samples at ≤ -20℃.

Serum – Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 10 minutes at 1,000 × g. Remove serum and assay immediately or aliquot and store samples at ≤ -20℃.

Plasma – Collect plasma using EDTA, citrate or heparin as anticoagulant. Centrifuge at 1,000 × g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20℃.

Other biological samples might be suitable for use in the assay. Cell culture supernates, serum and plasma were tested with this assay.

Note: Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens.
Samples should be aliquoted and must be stored frozen at -20℃ to avoid loss of bioactive ET-1. If samples are to be run within 24 hours, they may be stored at 2 to 8℃.
Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

REAGENT PREPARATION

Bring all reagents and samples to room temperature before use.
If crystals form in the Buffer Concentrates, warm and gently stir them until completely dissolved.

Washing Buffer (1×)

Pour entire contents (50 mL) of the **Washing Buffer (20×)** into a clean 1000 mL graduated cylinder. Bring to final volume of 1000 mL with pure or deionized water.
Mix gently to avoid foaming.
Transfer to a clean wash bottle and store at 2 to 25℃. Washing Buffer (1×) is stable for 30 days.

Assay Buffer (1×)

Pour the entire contents (10 mL) of the **Assay Buffer (10×)** into a clean 100 mL graduated cylinder. Bring to final volume of 100 mL with distilled water. Mix gently to avoid foaming.
Store at 2 to 8℃. Assay Buffer (1×) is stable for 30 days.

ET-1 Conjugate

Mix well prior to making dilutions.
Make a **1：100** dilution of the concentrated **ET-1 Conjugate** solution with Assay Buffer (1×) in a clean plastic tube as needed.
The diluted ET-1 Conjugate should be used within 30 minutes after dilution.

Streptavidin-HRP

Mix well prior to making dilutions.
Make a **1：100** dilution of the concentrated **Streptavidin-HRP** solution with Assay Buffer (1×) in a clean plastic tube as needed.
The diluted Streptavidin-HRP should be used within 30 minutes after dilution.

Sample Dilution

If your samples have high ET-1 content, dilute serum/plasma samples with Assay Buffer (1×). For cell culture supernates, dilute with cell culture medium without serum.

ET-1 Standard

Reconstitute **Human ET-1 Standard** by addition of distilled water. Reconstitution volume is stated on the label of the standard vial. Swirl or mix gently to insure complete and homogeneous solubilization (concentration of reconstituted standard = 40 pg/mL).
Allow the standard to reconstitute for 10 - 30 minutes. Mix well prior to making dilutions.
Use polypropylene tubes.

For serum/plasma samples, mixing *concentrated ET-1 standard* (230 µL) with 230 µL of *Standard Diluent* creates the high standard (20 pg/mL). Pipette 230 µL of *Standard Diluent* into each tube. Use the high standard to produce a 1:1 dilution series (scheme below). Mix each tube thoroughly before the next transfer. *Standard Diluent* serves as the zero standard (0 pg/mL).

For cell culture supernates, mixing *concentrated ET-1 standard* (230 µL) with 230 µL of cell culture medium without serum creates the high standard (20 pg/mL). Pipette 230 µL of cell culture medium without serum into each tube. Use the high standard to produce a 1:1 dilution series. Mix each tube thoroughly before the next transfer. Cell culture medium without serum serves as the zero standard (0 pg/mL).

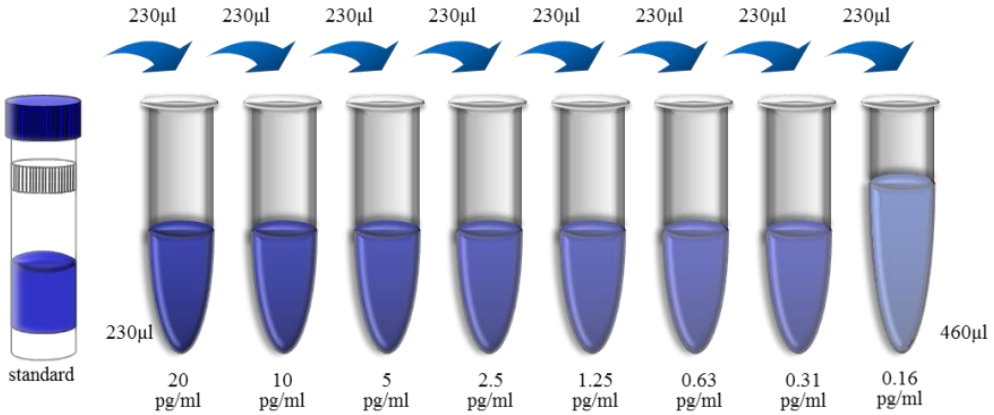


PLATE SETUP

Each plate or set of strips must contain a minimum of two Blanks, two non-specific binding wells (NSB), two maximum binding wells (B_0), and an eight point standard curve run in duplicate.

Note: Each assay must contain this minimum configuration in order to ensure accurate and reproducible results. Each sample should be assayed at two dilutions and each dilution should be assayed in duplicate. For statistical purposes, we recommend assaying samples in triplicate.

A suggested plate format and pipetting summary are shown below. The user may vary the location and type of wells present as necessary for each particular experiment.

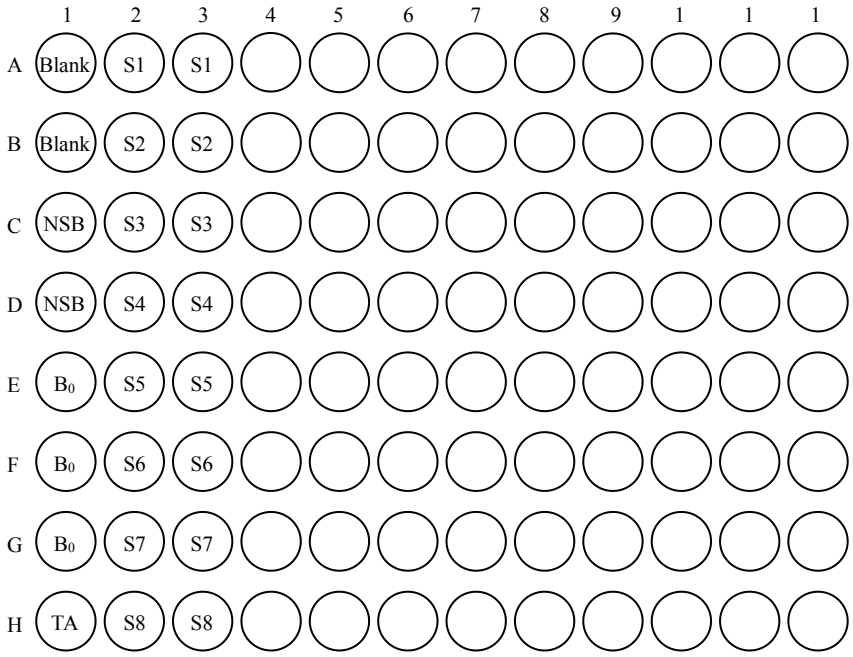
Component / Well	Standard Diluent	Assay Buffer (1×)	ET-1 Standard	Sample	ET-1 Conjugate	SA-HRP
Blank	-	-	-	-	-	-
NSB	100 µL	50 µL	-	-	-	100 µL
B_0	100 µL	-	-	-	50 µL	100 µL
TA	-	-	-	-	-	2 µL (at develop step)
Standard	-	-	100 µL	-	50 µL	100 µL
Sample	-	-	-	100 µL	50 µL	100 µL

Blank: background absorbance.

NSB (Non-Specific Binding): non-immunological binding of the conjugate to the well.

TA (Total Activity): total enzymatic activity of the conjugate.

B_0 (Maximum Binding): maximum amount of the conjugate that the antibody can bind in the absence of free analyte.



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use.

1. Prepare all reagents including microplate, samples, standards and working solution as described in the previous sections.
2. Remove excess microplate strips and return them to the foil pouch containing the desiccant pack, and reseal for further use.
3. Add 300 µL *Washing Buffer (1×)* per well, and allow it for about 30 seconds before aspiration. Soaking is highly recommended to obtain a good test performance. Empty wells and tap microwell strips on absorbent pad or paper towel to remove excess *Washing Buffer (1×)*. Use the microwell strips immediately after washing. **Do not allow wells to dry.**
4. Add 100 µL of serial diluted *Standard* to Standard wells in duplicate. Add 100 µL of *Standard Diluent* (For Serum/Plasma sample) or *Cell culture medium* (For cell culture supernates) to NSB and B_0 wells. (The dilution refers to the Sample Preparation on Page 6).
5. Add 100 µL of prepared samples to Sample wells.
6. Add 50 µL of *Assay Buffer (1×)* to NSB wells. Add 50 µL of diluted *ET-1 Conjugate* to each well **except Blank, NSB and TA wells**. Ensure reagent addition in step 4, 5 and 6 is uninterrupted and completed within 15 minutes.
7. Cover with an adhesive strip. Incubate at room temperature (22 to 28℃) for 1 hour on a microplate shaker set at 300 rpm.
8. Aspirate each well and wash, repeating the process five times for a total six washes. Wash by filling each well with 300 µL *Washing Buffer (1×)*. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Washing Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
9. Add 100 µL of diluted *Streptavidin-HRP* to each well **except Blank and TA wells**.
10. Cover with an adhesive strip. Incubate at room temperature (22 to 28℃) for 30 minutes on a microplate shaker set at 300 rpm.

11. Repeat aspiration/wash as in step 8.
12. Add 2 µL of diluted *Streptavidin-HRP* to TA well.
13. Add 100 µL of *Substrate Solution* to each well. Incubate for 5 - 30 minutes at room temperature (22 to 28℃). Protect from light.
14. Add 100 µL of *Stop Solution* to each well. The color will turn yellow. If the color in the well is green or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
15. Measure the optical density value within 30 minutes by microplate reader set to 450 nm. If wavelength correction is available, set to 570 nm or 630 nm. If wavelength correction is not available, subtract readings at 570 nm or 630 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Reading directly at 450 nm without correction may generate higher concentration than true value.

CALCULATION OF RESULTS

Average the duplicate readings for each standards (including B_0) and sample, and subtract the average NSB optical density (O.D.).

% B/B_0 can be calculated by dividing the corrected O.D. for each standard or sample by the corrected B_0 O.D. and multiplying by 100.

Plot % B/B_0 for standards S1 - S8 versus ET-1 concentration using linear (y) and log (x) axes and draw the best-fit curve through the plotted points (e.g. 4-parameter logistic).

Calculate the concentration of ET-1 corresponding to the mean absorbance from the standard curve.

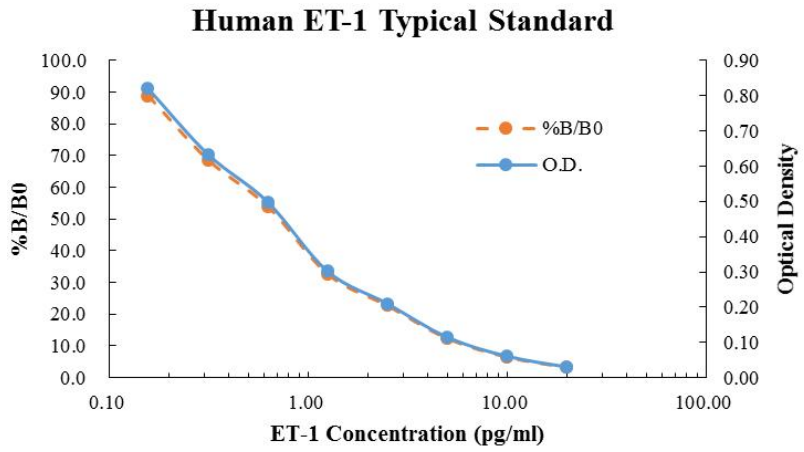
Note: Blank and TA values are not used in the standard curve calculations. Rather, they are used as diagnostic tools.

The finally concentration of top standard is 20 pg/mL. If sample have been diluted by other means, the concentration read from the standard curve must be multiplied by the appropriate dilution factor.

TYPICAL DATA

A standard curve must be run within each assay. This standard curve is provided for demonstration only.

pg/mL	O.D.		Average	Corrected	%B/B$_0$
NSB	0.017	0.018	0.018	-	-
B_0	0.938	0.946	0.942	0.924	-
0.16	0.841	0.836	0.839	0.821	88.805
0.31	0.680	0.624	0.652	0.635	68.632
0.63	0.532	0.501	0.517	0.499	53.975
1.25	0.331	0.309	0.320	0.303	32.720
2.50	0.233	0.223	0.228	0.211	22.769
5.00	0.140	0.126	0.133	0.116	12.493
10.00	0.082	0.076	0.079	0.062	6.652
20.00	0.048	0.049	0.049	0.031	3.353



SENSITIVITY

The minimum detectable dose (MDD) of ET-1 is typically less than 0.11 pg/mL.
The MDD was determined by subtracting two standard deviations to the mean optical density value of ten zero standard replicates and calculating the corresponding concentration.

PRECISION

Intra-assay Precision (Precision within an assay)
Three serum-based and buffer-based samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)
Three serum-based and buffer-based samples of known concentration were tested in six separate assays to assess inter-assay precision.

	Intra-assay precision				Inter-assay precision		
Sample	1	2	3		1	2	3
n	20	20	20		6	6	6
Mean (pg/mL)	0.65	2.61	9.92		0.72	2.71	10.23
Standard deviation	0.04	0.11	0.31		0.03	0.19	0.50
CV (%)	6.15	4.21	3.13		4.17	7.01	4.89

RECOVERY

The spike recovery was evaluated by spiking 3 levels of ET-1 into five health serum samples. The un-spiked serum was used as blank in these experiments.
The recovery ranged from 90 % to 108 % with an overall mean recovery of 98 %.

LINEARITY

To assess the linearity of the assay, five samples were spiked with high concentration of ET-1 in serum and diluted with Standard Diluent to produce samples with values within the dynamic range of the assay.

	Average (%)	Range (%)
1:2	91	80 - 107
1:4	100	93 - 115
1:8	95	87 - 110
1:16	89	82 - 105

CALIBRATION

This immunoassay is calibrated against a highly purified recombinant ET-1 produced at MultiSciences.

SAMPLE VALUES

Serum/Plasma - Thirty samples from apparently healthy volunteers/mice/rats were evaluated for the presence of ET-1 in this assay.

Sample Matrix	Number of Samples Evaluated	Range (ng/mL)	Detectable (%)	Mean of Detectable (ng/mL)
Serum	30	1.6 - 3.1	100	2.4

Note: The sample range is non-physiological range. The sample range of healthy human/mice/rats will difference according to geographical, ethic, sample preparation, and testing personnel, equipment varies. The above information is only reference.

SPECIFICITY

Human	Mouse	Rat
Big Endothelin-1 (aa 16-38) Big Endothelin-2 Sarafotoxin S6b Sarafotoxin S6c	Big Endothelin-39	Big Endothelin-39