Product Manual

Big ENDOTHELIN-1

Catalog #: ENZ-KIT151

ENZYME IMMUNOASSAY FOR THE QUANTITATIVE DETERMINATION OF HUMAN BIG ENDOTHELIN-1 IN SERUM, CITRATE PLASMA, EDTA PLASMA OR HEPARIN PLASMA.

1 x 96 wells

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Product Manual

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Please read entire booklet before proceeding with the assay.

Carefully note the handling and storage conditions of each kit component.

Please contact Enzo Life Sciences Technical Support if necessary.

INTRODUCTION

BIG ENDOTHELIN-1 (BigET) is a peptide of 38 amino acids and is the precursor of Endothelin-1 (ET), represented by amino acids 1-21 (http://www.uniprot.org/uniprot/P05305). ET is a potent vasoconstrictor and is produced by vascular endothelial cells. Accordingly it has a wide tissue distribution (http://www.ncbi.nlm.nih.gov/UniGene/ESTProfileViewer.cgi?uglist=Hs.511899). The cleavage of BigET by Endothelin Converting Enzyme (ECE) leads to ET and to a C-terminal fragment. Both BigET and ET are strong independent predictors of survival in patients with congestive heart failure, and identify a population with a very high risk of mortality. The half-life of ET (1-21) in plasma is less than one minute, whereas clearance of BigET is much slower. BigET can therefore be determined more easily.

Areas of Interest

- prognostic value in heart failure and acute myocardial infarction renal insufficiency
- during and after graft rejection atherosclerosis
- pulmonary hypertension and scleroderma

MATERIALS SUPPLIED

CONT	KIT COMPONENTS	QUANTITY
PLATE	Polyclonal sheep anti human BIG ENDOTHELIN-1 antibody coated microtiter strips in stripholder packed in aluminum bag with desiccant	12 x 8 tests
WASHBUF	Wash buffer concentrate 20x, natural cap	1 x 50 mL
AB	Monoclonal mouse anti-human BIG ENDOTHELIN-1 antibody, biotin labelled, red dye, green cap, ready-to-use	1 x 18 mL
STD	Standards human sera, synthetic human BIG ENDOTHELIN-1 (0, 0.10, 0.20, 0.40, 1, 3 pmol/l), lyophilized, white caps	6 vials
CTRL	Control human serum, synthetic human BIG ENDOTHELIN-1, lyophilized, yellow cap, exact concentration after reconstitution see label	1 vial
CONJ	Conjugate, (streptavidin-HRPO), amber cap, ready-to-use	1 x 22 mL
SUB	Substrate, (TMB solution), blue cap, ready-to-use	1 x 22 mL
STOP	Stop solution, white cap, ready-to-use	1 x 7 mL

ADDITIONAL MATERIALS ADDED

- 2 self-adhesive plastic film
- Instruction manual for use

ADDITIONAL MATERIALS AND EQUIPMENT NEEDED

- Precision pipettes calibrated to deliver 50 µL, 150 µL, 200 µL, 300 µL, 500 µL and disposable tips
- Distilled or deionized water
- Plate washer is recommended for washing, alternative multichannel pipette or manifold dispenser
- Refrigerator with 4°C (2-8°C)
- ELISA reader for absorbance at 450nm (reference 630nm) Graph paper or software for calculation of results

REAGENTS AND SAMPLE PREPARATION

All reagents of the kit are stable at 4°C (2-8°C) until the expiry date stated on the label of each reagent.

Sample preparation:

Serum and plasma are suitable for use in this assay. Note that BigET levels can differ between serum and plasma (see Assay Characteristics, page 7). Therefore don’t change sample type during studies. We recommend to separate plasma or serum by centrifugation as soon as possible (at least within one day) e.g. 20 min at 2,000 x g, preferably at 4°C (2-8°C). Aliquot the acquired plasma or serum samples and store them at -25°C or lower. All samples should undergo only 4 freeze-thaw cycles. Lipemic or hemolyzed samples may give erroneous results. Samples should be mixed well before assaying. Samples measuring OD above the highest STD can be diluted with the same BigET negative sample matrix, e.g. for serum samples use STD 0pmol/l or BigET negative human serum. We recommend duplicates for all values.

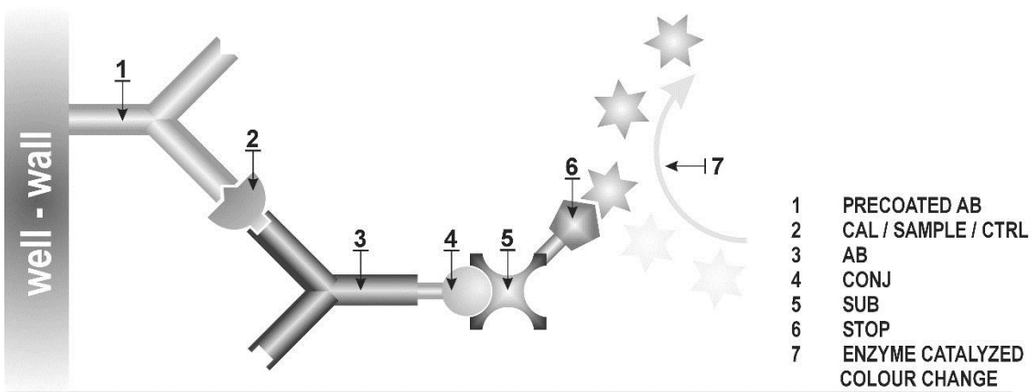
Reconstitution/Handling:

WASHBUF (Wash buffer): Dilute the concentrate 1:20 (1+19) eg. 50 mL concentrate + 950 mL distilled water. Crystals in the buffer concentrate will dissolve at room temperature. Buffer is stable at 4°C (2-8°C) until expiry date stated on label. Use only diluted WASHBUF (Wash buffer) for the assay performance.

STD (Standard): Pipette 500 µL of distilled or deionized water into each vial. Leave at room temperature (18-24°C) for 10 min. Swirl gently. The standard concentration is printed on the label. Reconstituted standard is stable at -25°C or lower until expiry date. Avoid freeze-thaw cycles.

CTRL (Control): Pipette 500 µL of distilled or deionized water to the vial. Leave at room temperature (18-24°C) for 10 min. Swirl gently. The final concentration is stated on the label. Reconstituted control is stable at -25°C or lower until expiry date stated on label. Avoid freeze-thaw cycles.

PRINCIPLE OF THE ASSAY



ASSAY PROTOCOL

All reagents and samples must be at room temperature (18-24°C) before use in the assay.

Mark position for BLANK/STD (Standards) /SAMPLE/CTRL (Control) on the supplied protocol sheet.

Take microtiter strips out of the aluminum bag, reserve a minimum of one well as Blank. Store unused strips with desiccant at 2-8°C in the aluminum bag. Strips are stable until expiry date stated on the label.

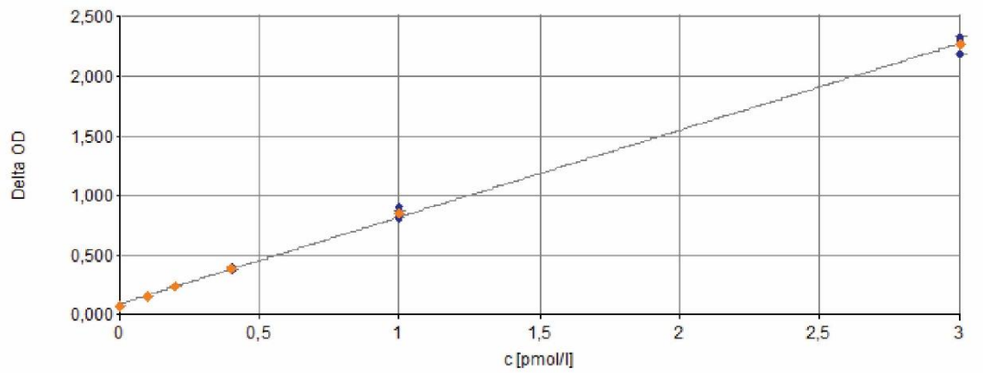
1. Add 50 µL STD/SAMPLE/CTRL (Standard, white caps/Sample/Control, yellow cap) in duplicate into respective well, except blank.
2. Add 150 µL AB (biotinylated anti-BigET antibody, green cap, red dye) into each well, except blank, swirl gently.
3. **Cover tightly and incubate 4 hours at room temperature (18-24°C) in the dark.**
4. Aspirate and wash wells 5x with 300 µL diluted WASHBUF (Wash buffer). Remove remaining WASHBUF by hitting plate against paper towel after the last wash.
5. Add 200 µL CONJ (streptavidin-HRPO, amber cap) into each well.
6. **Cover tightly and incubate 1 hour at room temperature (18-24°C) in the dark**.
7. Aspirate and wash wells 5x with 300 µL diluted WASHBUF (Wash buffer). Remove remaining WASHBUF by hitting plate against paper towel after the last wash.
8. Add 200 µL SUB (Substrate, blue cap) into each well.
9. **Incubate for 30 minutes at room temperature (18-24°C) in the dark.**
10. Add 50 µL STOP (Stop solution, white cap) into each well, shake well.
11. Measure absorbance immediately at 450 nm with reference 630 nm, if available.

CALCULATION OF RESULTS

Read the optical density (OD) of all wells on a plate reader using 450 nm wavelength (correction wavelength 630 nm). Subtract the blank OD from the values of STD, CTRL and sample. Construct the standard curve from the OD values of the STD. Use software or graph paper. Obtain sample concentration from this standard curve. The assay was evaluated with a 4 PL algorithm. Different curve fitting methods need to be evaluated by the user. Respective dilution factors have to be considered.

If the OD of highest STD is outside the measuring range of photometer can be measured at 405 nm (correction wavelength 630 nm).

TYPICAL STANDARD-CURVE



The quality control protocol supplied with the kit shows the results of the final release QC for each kit at production date. Data for OD obtained by customers may differ due to various influences and/or due to the normal decrease of signal intensity during shelf life. However, this does not affect validity of results as long as an OD of 1.00 or higher is obtained for the standard with the highest concentration and the control value is in range (target range see label).

ASSAY CHARACTERISTICS

Values from apparently healthy individuals:	Serum: Median: 0.09 pmol/l (n=41). Each laboratory should establish its own reference range for the samples under investigation. Do not change sample type during studies.
Standard range:	0-3 pmol/l
Conversion factorpg/mL topmol/l:	1 pg/mL = 0.2335 pmol/l (MW: 4.283 kDa)
Sample volume:	50 µL human serum or plasma (Citrate, EDTA or Heparin)
Detection limit:	(0pmol/l + 3 SD): 0.02 pmol/l
Incubation time:	4 h / 1 h / 30 min
Cross reactivity:	ET1/2/3 (1-21): <1%, ET2 (1-37): <1%, ET1/2 (1-38): <1%, porcine BigET (1-39): 21%, BigET1/2 (22-38) : <1%, BigET2 (22-37) : <1%, rat BigET1 (1-39): 10%, Sarafotoxin: <1%

PRECISION

Intra-Assay: 2 samples of known concentrations were tested 5 times in 1 assay.

Inter-Assay: 2 samples of known concentrations were tested 10 times within 3 assays each by a different operator.

Intra-Assay (n=5)	Sample 1	Sample 2	Inter-Assay (n=10)	Sample 1	Sample 2
Mean (pmol/l)	0.20	1.00	Mean (pmol/l)	0.20	1.00
SD (pmol/l)	0.003	0.048	SD (pmol/l)	0.009	0.041
CV%	2%	5%	CV%	4%	4%

TECHNICAL HINTS

- Do not mix or substitute reagents with those from other lots or sources.
- Do not mix stoppers and caps from different reagents or use reagents between lots.
- Do not use reagents beyond expiration date.
- Protect reagents from direct sunlight.
- Substrate solution should remain colorless until added to the plate.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Avoid foaming when mixing reagents.

PRECAUTIONS

- All test components of human source were tested with 3rd generation tests against HIV-Ab and HBsAg; and were found negative. Nevertheless, they should be handled and disposed as if they were infectious.
- Liquid reagents contain ≤0.1% Proclin 300 as preservative. Proclin 300 is not toxic in concentrations used in this kit. It may cause allergic skin reactions – avoid contact with skin or eyes.
- Do not pipette by mouth.
- Do not eat, drink, smoke or apply cosmetics where reagents are used.
- Wear gloves, glasses and lab jacket while performing this assay.
- Sulfuric acid is irritating to eyes and skin. Avoid contact with skin and mucous. Irritations are possible – flush with water if contact occurs!

Handle with care

LITERATURE

1. Burg M et al., Depression Predicts Elevated Endothelin-1 in Patients With Coronary Artery Disease. Psychosom Med (2011), 73: 2-6
2. Van Beneden R et al., Superiority of BIG ENDOTHELIN-1 and endothelin-1 over natriuretic peptides in predicting survival in severe congestive heart failure: a 7-year follow-up study. J Card Fail (2004), 10(6): 490-495
3. Lockowandt U et al., Plasma levels and vascular effects of endothelin and big endothelin in patients with stable and unstable angina pectoris undergoing coronary bypass grafting. Eur J Cardiothorac Surg (2002), 21(2):218-223
4. Frey B et al., Prognostic value of hemodynamic vs big endothelin measurements during long-term therapy in advanced heart failure patients. Chest (2000), 117(6):1713-1719
5. Arun C. et al., The role of BIG ENDOTHELIN-1 in colorectal cancer. Int J Biol Markers (2002), 17(4):268-274

ASSAY PROTOCOL AND CHECKLIST

PREPARATION OF REAGENTS:

- Bring all reagents to room temperature (18-24°C).
- Prepare reagents and samples as instructed.
- Bring unused and prepared components to the storage temperature mentioned in the package insert.
- Take microtiter strips out of the alu bag and mark positions on the protocol sheet.

TEST PROCEDURE:

- **Step 1**: Add 50 µL STD/SAMPLE/CTRL (standard/sample/control) in duplicate into respective wells except blank.
- **Step 2**: Add 150 µL AB (biotinylated anti BigET-1 antibody) into all wells except blank, swirl gently.
- **Step 3: Cover tightly and incubate for 4 hours at room temperature (18-24°C) in the dark.**
- **Step 4**: Aspirate and wash wells with 300 µL WASHBUF (wash buffer) five times. Remove remaining buffer by hitting plate against paper towel.
- **Step 5**: Add 200 µL CONJ (streptavidin-HRPO, amber cap) into all wells except blank.
- **Step 6: Cover tightly and incubate for 1 hour at room temperature (18-24°C) in the dark.**
- **Step 7**: Aspirate and wash wells with 300 µL WASHBUF (wash buffer) five times. Remove remaining buffer by hitting plate against paper towel.
- **Step 8**: Add 200 µL SUB (substrate, blue cap) into each well.
- **Step 9: Incubate for 30 minutes at room temperature (18-24°C) in the dark**.
- **Step 10**: Add 50 µL STOP (Stop solution, white cap) into each well.
- **Step 11**: Read Optical Density at 450 nm with reference 630 nm, if available.

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