PromoKine

Human Big Endothelin ELISA Kit

Instruction Manual

Cat.No. PK-EL-KB20082

Contents

Intended Use

This PromoKine ELISA Kit is a sandwich ELISA intended for the quantitative determination of human Big Endothelin in serum, EDTA-plasma, urine and cell culture supernatants. **It is for research use only.**

Introduction

Big Endothelin-1 (Big ET), a small 38-amino-acid peptide, is the biological precursor of Endothelin (1-21), the most potent vasoconstrictor known today. Various cell types including vascular endothelial cells and non-vascular cells (eg. mesanglial, kidney and epithelial cells), produce Endothelin. Cleavage of Big ET by the Endothelin Converting Enzyme (ECE leads to the active ET (1-21) and to the C-terminal fragment (22-38). The physiological importance of cleavage of Big ET is indicated by the reported 140-fold increase in vasoconstrictor activity upon cleavage to ET-1, although both peptides can be determined in about equimolar concentrations in plasma. It was demonstrated that the half-life of ET (1-21) in plasma is less than one minute, whereas clearance of Big ET is much slower.

Possible Indications

- Prognostic value in heart failure and acute myocardial infarction
- Renal insufficiency
- During and after graft rejection
- Increased plasma levels: hypochelesterolemia
- Artherosclerosis
- Pulmonary hypertension and scleroderma

Material Supplied

Content	Kit Components	Quantity
PLATE	Polyclonal sheep anti Big Endothelin-1 antibody coated microtiter strips in stripholder packed in alu bag with desiccant	12 x 8 tests
WASHBUF	Wash buffer concentrate 20x , natural cap	1 x 50 ml
STD	Standards human sera, synthetic human Big Endothelin-1 (0, 0.10, 0.20, 0.40, 1, 3 pmol/l), lyophilised, white caps	6 vials lyophilised
CTRL	Control human serum, synthetic human Big Endothelin-1, lyophilised, yellow cap, exact concentration after reconstitution see label	1 vial lyophilised
CONJ	Conjugate, (streptavidin-HRPO), amber cap, ready to use	1 x 22 ml
AB	Monoclonal mouse anti human Big Endothelin-1 antibody, biotin labelled, red dye, green cap, ready to use	1 x 18 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

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

Material and Equipment Required but not Provided

- Precision pipettes calibrated to deliver 50-500 µl and disposable tips
- Elisa plate reader for absorbance at 450 nm (reference 630 nm)
- Graph paper or software for calculation of results
- Plate washer is recommended for washing, alternative multichannel pipette or manifold dispenser
- Distilled or deionised water

Preparation of Reagents and Samples

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

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. The undiluted WASHBUF is stable at 4°C (2-8°C) until expiry date stated on label. The diluted WASHBUF is stable up to one month at 4°C (2-8°C). Only use diluted WASHBUF when performing the assay.

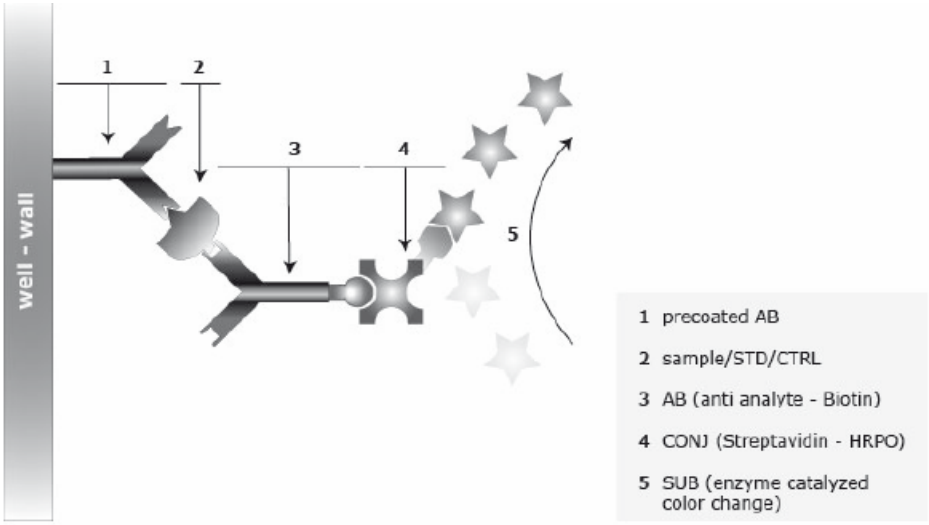
STD (Standards) + CTRL (Control): Pipette 500 Kl of distilled or deionised water into each vial. Leave at room temperature (18-24°C) for 10 min. Swirl gently. The exact concentration is printed on the label. Reconstituted STDs and CTRL are stable at -25°C or lower until expiry date. Avoid freeze-thaw cycles.

Sample preparation:

Serum and plasma are suitable for use in this assay. Note that BigET levels can differ between serum and plasma therefore don't change sample type during studies. We recommend to separate plasma or serum by centrifugation, e.g. 20 minutes at 2,000 x g, preferably at 4°C (2-8°C), as soon as possible but within 2 hours after sample collection.

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 STD1 (0 pmol/l) or BigET negative human serum. We recommend duplicates for all values.

Principle of the assay.



Assay Protocol

- All reagents and samples must be at room temperature (18-24°C) before using in the assay
- Mark position for BLANK/STD (Standards)/SAMPLE/CTRL (Control)
- Take microtiter strips out of the aluminium bag. Store unused strips with desiccant at 4°C (2-8°C) in the aluminium bag. Strips are stable until expiry date stated on the label.

Protocol

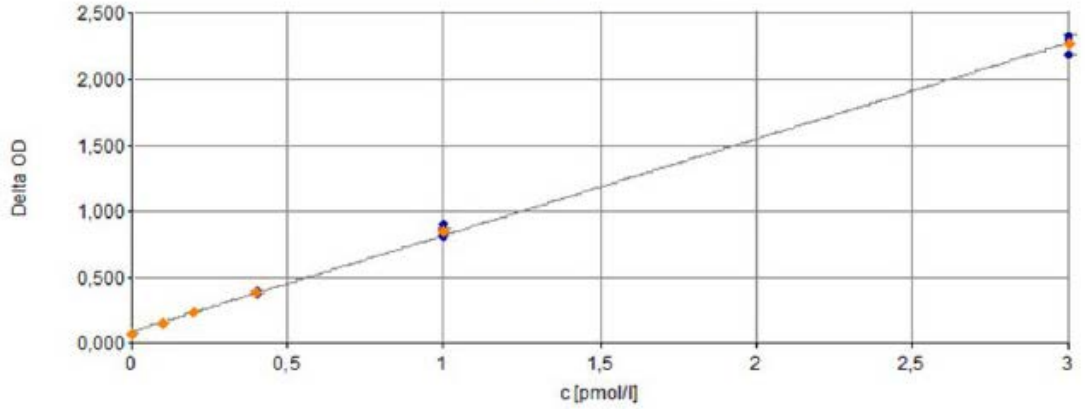
1. Add 50 Kl STD/SAMPLE/CTRL (Standard, white caps/Sample/Control, yellow cap) in duplicate into respective well.
2. Add 150 Kl AB (biotinylated anti BigET antibody, green cap, red dye) into each well, swirl gently.
3. Cover tightly and incubate 4 hours at room temperature (18-24°C) in the dark.
4. Aspirate and wash wells 5x with 300 Kl diluted WASHBUF (Wash buffer). Remove remaining WASHBUF by hitting plate against paper towel after the last wash.
5. Add 200 Kl 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 Kl diluted WASHBUF (Wash buffer). Remove remaining WASHBUF by hitting plate against paper towel after the last wash.
8. Add 200 Kl SUB (Substrate, blue cap) into each well.
9. Incubate for 30 minutes at room temperature (18-24°C) in the dark.
10. Add 50 Kl 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).

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 4PL algorithm. Different curve fitting methods need to be evaluated by the user. Respective dilution factors have to be considered. If the OD of the highest STD is outside the measuring range of photometer plate can be re-measured at 405nm (correction wavelength 630 nm).

Example typical STD 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

Method:	Sandwich ELISA, HRP/TMB, 12x8-well strips		
Sample type:	Serum, EDTA plasma, heparin plasma, and citrate plasma		
Standard range:	0 to 3 pmol/l (6 standards and 1 control in a human serum matrix)		
Conversion factor:	1 pg/ml = 0.2335 pmol/l (MW: 4.283 kDa)		
Sample volume:	50 Kl / well		
Incubation time:	4 h / 1 h / 30 min		
Sensitivity:	LOD: (0 pmol/l + 3 SD): 0.02 pmol/l; LLOQ: 0.03 pmol/l		
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 (n=5) ≤ 5%, Inter-assay (n=10) ≤ 4%		
Spike/Recovery (average recovery spiked with 1 pmol/l rec. BigET):	Serum (n=14) = 100%	Heparin plasma (n=3) = 97%	
	EDTA plasma (n=3) = 100%	Citrate plasma (n=3) = 98%	
Dilution linearity (average recovery of expected BigET after a 1+1 and 1+3 dilution):	Dilution:	1+1	1+3
	Serum (n=8)	90%	96%
	EDTA plasma (n=4)	110%	104%
Values from apparently healthy individuals:	Median serum (n=41) = 0.09 pmol/l Each laboratory should establish its own reference range for the samples under investigation. Do not change sample type during the study.		

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 by different operators.

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 colourless 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.

All liquid reagents contain 0.01% 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.
- Avoid all contact with the reagents by using gloves.
- Sulfuric acid is irritating to eyes and skin. Flush with water if contact occurs. Avoid contact with skin and mucous. Irritations are possible - Flush with water after contact!!

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

Ordering Information

Product Name	Size	Catalog Number
Big Endothelin ELISA Kit, human	96 Tests	PK-EL-KB20082

For in vitro research use only.
Not for diagnostic or therapeutic procedures.

PromoCell GmbH
Sickingenstr. 63/65
69126 Heidelberg
Germany

USA / Canada
Phone: 1 – 866 – 251 – 2860 (toll free)
Fax: 1 – 866 – 827 – 9219 (toll free)

Deutschland
Telefon: 0800 – 776 66 23 (gebührenfrei)
Fax: 0800 – 100 83 06 (gebührenfrei)

France
Téléphone: 0800 90 93 32 (ligne verte)
Téléfax: 0800 90 27 36 (ligne verte)

United Kingdom
Phone: 0800 – 96 03 33 (toll free)
Fax: 0800 – 169 85 54 (toll free)

Other Countries
Phone: +49 6221 – 649 34 0
Fax: +49 6221 – 649 34 40

Email: info@promokine.info