# Human Endothelin (1-21) ELISA Kit

## Instruction Manual

PromoKine

# Contents





## Intended Use

This PromoKine ELISA Kit is a sandwich ELISA intended for the quantitative determination of human Endothelin (1-21) in serum, EDTA-plasma, urine and cell culture supernatants. **It is for research use only.**

## Introduction

Cleavage of Big Endothelin by a membrane-bound metalloproteinase, the Endothelin Converting Enzyme (ECE) leads to the active ET (1-21), a potent vasoconstrictor and to the biological inactive C-terminal fragment (22-38). The half-life of ET in the plasma is less than one minute, whereas clearance of Big ET is much slower. Endothelin has been identified in a variety of tissues, including lung, kidney, brain, pituitary and peripheral endocrine tissues and placenta.The biological role of ET extends beyond regulating vascular tone also in its growth regulatory properties. The peptide interacts in an autocrine/paracrine manner with specific ET receptors found on numerous cells, including smooth muscle cells, myoctyes, and fibroblasts.

*Possible Indications*

- Heart failure and acute myocardial infarction
- Oncology
- Marker for endothelial dysfunction, liver damage and renal disease
- Hypertension

## Material Supplied

| Content | Kit Components | Quantity |
|---|---|---|
| PLATE | Polyclonal anti Endothelin antibody, microtiter plates strips in stripholder packed in alubag with desiccant | 12 x 8 tests |
| WASHBUF | Wash buffer concentrate 20x | 1 x 50 ml |
| AB | Detection antibody, monoclonal mouse anti Endothelin antibody, ready to use | 1 x 22 ml |
| STD | Standards ( 0-10 fmol/ml), synthetic human Endothelin-1 (1-21) in human plasma, white caps, lyophilised | 6 vials lyophilised |
| CTRL | Controls, synthetic human Endothelin-1 (1-21) in human plasma, yellow caps, lyophilised, exact concentration after reconstitution see label | 2 vials lyophilised |
| CONJ | Conjugate, (anti mouse IgG antibody-HRPO), ready to use | 1 x 22 ml |
| SUB | Substrate (TMB solution), ready to use | 1 x 22 ml |
| STOP | Stop Solution, ready to use | 1 x 7 ml |
| ET-STOCK | Endothelin Stock, synthetic human Endothelin-1 (1-21), lyophilised, red cap, exact concentration after reconstitution see label | 1 vial lyophilised |

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



## Material and Equipment Required but not Provided

- Precision pipettes calibrated to deliver 50-1000 µl and disposable tips
- Elisa reader for absorbance at 450 nm (reference 620 nm), Graph paper or software for calculation of results
- Distilled or deionised water

## Preparation of Reagents and Samples

*Reconstitute as follows:*

- STD (Standards, white caps) in 0.5ml distilled water, at room temperature (18-26°C) for 30 minutes, shake well.
- Reconstituted standards are stable at –20°C or –70°C until expiry date stated on label. Avoid repeated freeze-thaw cycles!
- CTRL (Controls, yellow caps) in 0.5 ml distilled water at room temperature (18-26°C) for 30 minutes, stable at –20°C or –70°C until expiry date stated on label, avoid freeze-thaw cycles.
- WASHBUF (Wash buffer) dilute the concentrate 1:20 with distilled water (50 ml concentrate + 950 ml distilled water). Crystals in the buffer concentrate will dissolve at room temperature. Buffer is stable at 2-8°C until expiry date stated on label.
- ET-STOCK (Endothelin stock, red cap): Direct measurement of Endothelin in cell culture supernatants: Reconstitute in 2 ml cell culture medium at room temperature (18-26°C) for 30 min, shake well. The solution contains 10 fmol/ml Endothelin. Reconstituted ET stock is stable at -20°C or –70°C until expiry date stated on the label. Avoid repeated freeze-thaw cycles.

*Sample type:*

Serum, EDTA plasma, urine, saliva and cell culture supernatants are suitable for use in this assay. Don't change sample type during studies.

*Sample collection:*

Freshly collected EDTA plasma or serum is put on ice immediately and centrifuged within one day. Samples should be stored at –20°C, for long-term storage store at -70°C.

*Urine samples* can be used without any pre-treatment.

Avoid freeze-thaw cycles. Lipemic or hemolyzed samples may give erroneous results. Samples should be mixed well before assaying. We recommend duplicates for all values. If it is necessary to dilute samples with a high concentration please use 0.9% sodium chloride solution.

*For cell culture:*

**Do not use the plasma-standards (white caps) and controls (yellow cap)!**

- Prepare a serial dilution of the cell culture ET-STOCK (Endothelin-stock, red cap) with cell culture medium down to appr. 0.625 fmol/ml (e.g. 10 / 5 / 2.5 / 1.25 / 0.625 fmol/ml). Cell culture medium is used as a zero standard.
- Dilute cell culture supernatant according to the expected concentration with the culture medium. Dilution of supernatant is dependent on amount of Endothelin secreted by the respective cell type.



*For saliva:*

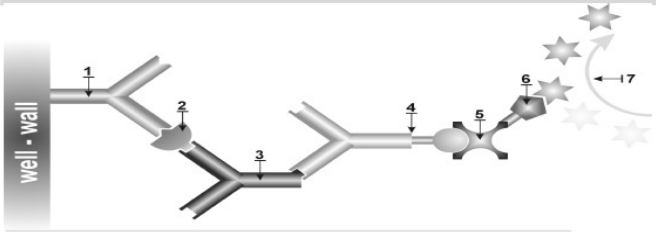
For sample collection we recommend Sarstedt Salivettes.

Sample collection:

Last meal or cigarette should have been consumed at least one hour before sample collection.

- Rinse mouth with water, wait 10 minutes.
- Put cotton roll of the Sarstedt Salivette tube into the mouth, chew for 30 sec., keep in mouth for two additional minutes.
- Place the cotton roll into the flat bottom upper tube of the Salivette, seal with the stopper and centrifuge for 3 min. at 1000 rcf (rotational centrifugal force = g).
- Remove the flat bottom tube from the Salivette and pipette the clear saliva from the bottom of the V-tube, aliquot and store at -20°C or –70°C.
- Use the clear saliva according to the assay protocol.

*6) PRINCIPLE OF THE ASSAY*



## Assay Protocol

- All reagents and samples must be at room temperature (18-26°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 alubag, take a minimum of one well as Blank. Store unused strips with desiccant at 2-8°C in the alubag. Strips are stable until expiry date stated on the label.
- Add 50 µl STD/SAMPLE/CTRL (Standard, white cap/Sample/Control, yellow cap) in duplicate into respective well, except blank.
- Add 200 µl AB (Detection antibody, green cap) into each well, except blank, swirl gently.
- **Cover tightly and incubate at room temperature (18-26°C) overnight (16-24 hours).**
- Aspirate and wash wells 5x with 300 µl diluted WASHBUF (Wash buffer), remove remaining WASHBUF by hitting plate against paper towel after the latest wash.
- Add 200 µl CONJ (Conjugate) into each well.
- **Cover tightly and incubate 1 hour at room temperature.**
- 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.
- Add 200 µl SUB (Substrate) into each well.
- **Incubate for 30 minutes at room temperature (18-26°C) in the dark.**
- Add 50 µl STOP (Stop solution) into each well, shake well.
- Measure absorbance immediately at 450 nm with reference 620 nm, if available.



## Calculation of Results

Subtract the blank extinction from all other values. Construct the Standard curve from the Standard values. Use commercially available software or graph paper. Obtain sample concentration from this calibration curve. The assay has been evaluated using a 4PL algorithm. Different curve fitting method needs to be evaluated by the user. Respective dilution factors have to be considered.

## Assay Characteristics

| | |
|---|---|
| **Reference data :** | A panel of 70 blood donors had a median of 0.26 fmol/ml) |
| | Each laboratory should establish its own reference data |
| **Standard range:** | 0 to 10 fmol/ml |
| **Sample volume:** | 50 µl human EDTA plasma, serum, urine, saliva or cell culture supernatant |
| **Detection Limit:** | (0 fmol/ml + 3 SD): 0.02 fmol/ml |
| **Incubation time:** | overnight / 1 h / 30 min |
| **Cross reactivity:** | ET(1-21): 100%, ET2 (1-21): 100%, ET3 (1-21): <5%, Big Endothelin (1-38): <1%, Big Endothelin ( 22-38): < 1% In normal human plasma samples ET-2 is estimated to be present at less than 20% of the ET-1 level. ET-3 is estimated to be present at 50% of the ET-1 level. Animal sera: |
| | Horse, cat, pig 100%; dog 66% and rat 79%, Mouse sera can not be measured in this ELISA. |

*Recovery*

| n = 4 | Spike 1 fmol/ml | Spike 5 fmol/ml |
|---|---|---|
| Recovery (fmol/ml) | 0.96 | 4,.3 |
| Recovery (%) | 96% | 86% |

## Precision

Intra-Assay (n=18)

| | | |
|---|---|---|
| Mean (fmol/l) | 2.02 | 7.01 |
| SD | 0.08 | 0.21 |
| CV | 4% | 3% |

Inter-Assay (n=24)

| | | |
|---|---|---|
| Mean (fmol/l) | 3.8 | 0.8 |
| SD | 0.2 | 0.04 |
| CV | 6% | 5% |

## 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



- For recovery experiments use cell culture STOCK (Big Endothelin stock) reconstituted in 2 ml distilled water
- Do not use lyophilised plasma standards for recovery experiments!
- 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


- "Plasma levels of endothelin, lipid peroxides and prostacyclin in diabetic patients with macroangiopathy" Migdalis IN et al.; Diabetes Res Clin Pract 2001 Nov; 54(2):129-36
- "Plasma endothelin in patients with acute aortic disease" Wagner A et al.; Resuscitation 2002 Apr; 53(1):71-6
- "Plasma endothelin-1 levels and clinical correlates in patients with chronic heart failure" [In Process Citation] Kinugawa T et al.; J Card Fail 2003 Aug; 9(4):318-24

## Assay Protocol and Checklist

- Bring all reagents to room temperature (18-26°C).
- Prepare reagents and samples as instructed.
- Take microtiter strips out of the alubag and mark positions on the protocol sheet.
- Add 50 µl STD/SAMPLE/CTRL (standard/sample/control) into all wells, except blank.
- Add 200 µl AB (Detection antibody) into each well, except blank, swirl gently.
- **Cover tightly and incubate at room temperature (18-26°C) over night (16-24 hours).**
- Aspirate and wash wells with 300 µl WASHBUF (Wash buffer) five times. Remove remaining buffer by hitting plate against paper towel.
- Add 200 µl CONJ (Conjugate) into each well.
- **Cover tightly and incubate at room temperature for 1 hour.**
- Aspirate and wash wells with 300 µl WASHBUF (Wash buffer) five times. Remove remaining buffer by hitting plate against paper towel.
- Add 200 µl SUB (Substrate) into each well.
- **Incubate at room temperature (18-26°C) for 30 minutes, in the dark.**
- Add 50 µl STOP (Stop solution) into each well.
- Read Optical Density at 450 nm with reference 620 nm, if available.



## Ordering Information

| Product Name | Product Description | Size | Catalog Number |
|---|---|---|---|
| Endothelin (1-21) ELISA Kit, human | Human Endothelin (1-21) ELISA Kit | 96 Tests | PK-EL-KB1140 |

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

**PromoCell GmbH**
Sickingenstr. 63/65
69126 Heidelberg
Germany

North America
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