Human Big Endothelin ELISA Kit

Cat. No.: DEIA6669
Pkg.Size: 96T

Intended use

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

General Description

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 (e.g., mesangial, 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.

Reagents And Materials Provided

1. PLATE Polyclonal sheep anti Big Endothelin-1 antibody coated microtiter strips in stripholder packed in alu bag with desiccant, 12 x 8 tests
2. WASHBUF, Wash buffer concentrate, 10x 1 x 100 ml
3. STD, Standards, synthetic human Big Endothelin-1 (1-38) in human plasma, lyophilised, white caps, exact concentration after reconstitution see label, 5 vials lyophilised
4. CTRL, Control, synthetic human Big Endothelin-1 (1-38) in human plasma, lyophilised, yellow cap, exact concentration after reconstitution see label, 1 vial lyophilised
5. CONJ, monoclonal anti Big Endothelin antibody HRP labelled, ready to use, 1 x 22 ml
6. SUB, Substrate (TMB solution), ready to use, 1 x 22 ml
7. STOP, Stop solution, ready to use, 1 x 7 ml
8. Cell culture STOCK, Big Endothelin-1 stock, synthetic human Big Endothelin–1 (1-38), lyophilised, amber vial with red cap, exact concentration after reconstitution see label, 1 vial lyophilised
9. 1 self-adhesive plastic films
10. Instruction manual for use

Materials Required But Not Supplied

1. Precision pipettes calibrated to deliver 50-1000 μl and disposable tips
2. Elisa reader for absorbance at 450 nm (reference 620 nm), Graph paper or software for calculation of results
3. Plate washer is recommended for washing
4. Distilled or deionised water

Specimen Collection And Handling

Sample type:
Serum, EDTA – plasma, urine and cell culture supernatants are suitable for use in this assay. Note that Big ET levels can be differ between serum and EDTA-plasma. Therefore 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 pretreatment.
All samples should undergo only 4 freeze-thaw cycles. Lipemic or hemolyzed samples may give erroneous results. Samples should be mixed well before assaying. We recommend duplicates for all values.

**For cell culture:**
Do not use the plasma-standards 1-5 (white caps) and control (yellow cap)!
Prepare a serial dilution of the cell culture -STOCK (Big Endothelin-1 stock) with cell culture medium down to appr. 0.6 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 Big ET secreted by the respective cell type.

**Reagent Preparation**

**Reconstitute as follows:**
STD (Standards, white caps) 1 to 5 in 0,5 ml distilled water at room temperature (18-26°C) for 20 minutes, shake well. Reconstituted standards are stable at –20°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 20 minutes, shake well. Reconstituted control is stable at –20°C until expire date stated on label. Avoid repeated freeze–thaw cycles.
WASHBUF (Wash buffer) dilute the concentrate 1:10 with distilled water. (100 ml concentrate + 900 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. Cell culture STOCK (Big Endothelin-1 stock, amber vial with red cap) in 2 ml of cell culture medium, at room temperature (18-26°C) for 20 minutes, shake well. The solution contains 10 fmol/ml Big Endothelin-1. Reconstituted standard is stable at –20°C until expiry date stated on the label. Avoid repeated freeze- thaw cycles!

**Assay Steps**

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 alu bag, take a minimum of one well as Blank. Store unused strips with desiccant at 2-8° C in the alu 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 200 μl CONJ into each well, except blank, swirl gently,
3. Cover tightly and incubate 4 hours at room temperature (18-26°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 SUB (Substrate) into each well
6. Incubate for 30 minutes at room temperature (18-26°C) in the dark
7. Add 50 μl STOP (Stop solution) into each well, shake well
8. Measure absorbance immediately at 450 nm with reference 620 nm, if available

**Calculation**
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 need to be evaluated by the user. Respective dilution factors have to be considered.

**Sensitivity**

(0 fmol/ml + 3 SD): 0,02-0,07fmol/ml

**Specificity**

ET1/2/3 (1-21): <1%, ET2 (1-37): <1%, ET1/2 (1-38): <1%, porcine Big ET (1-39): <16, Big ET-1/2 (22-38) <1%, Big ET-2 (22-37) <1%, Sarafotoxin <1%, rat BigET-1 (1-39) 10%

**Recovery**

3 plasma have been spiked with 3 levels of synthetic Big Endothelin-1:

<table>
<thead>
<tr>
<th>Recovery (fmol/ml)</th>
<th>n = 3</th>
<th>10 fmol/ml</th>
<th>5 fmol/ml</th>
<th>1 fmol/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recovery (%)</td>
<td>109%</td>
<td>94%</td>
<td>74%</td>
<td></td>
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</tbody>
</table>

**Reproducibility**

**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.

1. 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.
2. Do not pipette by mouth.
3. Do not eat, drink, smoke or apply cosmetics where reagents are used.
4. Avoid all contact with the reagents by using gloves.
5. Sulfuric acid is irritating to eyes and skin. Flush with water if contact occurs. Avoid contact with skin and mucous.
6. Irritations are possible - Flush with water after contact!!

REFERENCES