ENDOTHELIN (1-21) ELISA ASSAY KIT

ENZYME IMMUNOASSAY FOR THE QUANTITATIVE DETERMINATION OF HUMAN ENDOTHELIN (1-21) IN SERUM, EDTA PLASMA, URINE, SALIVA AND CELL CULTURE SUPERNATANTS.

CAT. NO. BI-20052. 12 X 8 TESTS

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

rev.no. 120216  (replacing 090701)

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1) 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, myocytes, and fibroblasts.

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

2) CONTENTS OF THE KIT

<table>
<thead>
<tr>
<th>CONT</th>
<th>KIT COMPONENTS</th>
<th>QUANTITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLATE</td>
<td>Polyclonal anti Endothelin antibody, microtiter plate strips in stripholder packed in aluminium bag with desiccant</td>
<td>12 x 8 tests</td>
</tr>
<tr>
<td>WASHBUF</td>
<td>Wash buffer concentrate 20x, natural cap</td>
<td>1 x 50 ml</td>
</tr>
<tr>
<td>AB</td>
<td>Detection antibody, monoclonal mouse anti Endothelin antibody, green cap, ready to use</td>
<td>1 x 22 ml</td>
</tr>
<tr>
<td>STD</td>
<td>Standards (0-10 fmol/ml), synthetic human Endothelin-1 (1-21) in human serum, white caps, lyophilised</td>
<td>6 vials lyophilised</td>
</tr>
<tr>
<td>CTRL</td>
<td>Controls, synthetic human Endothelin-1 (1-21) in human serum, yellow caps, lyophilised, exact concentration after reconstitution see label</td>
<td>2 vials lyophilised</td>
</tr>
<tr>
<td>CONJ</td>
<td>Conjugate (anti mouse IgG antibody-HRPO), amber cap, ready to use</td>
<td>1 x 22 ml</td>
</tr>
<tr>
<td>SUB</td>
<td>Substrate (TMB solution), blue cap, ready to use</td>
<td>1 x 22 ml</td>
</tr>
<tr>
<td>STOP</td>
<td>Stop Solution, white cap, ready to use</td>
<td>1 x 7 ml</td>
</tr>
<tr>
<td>ET-STOCK</td>
<td>Endothelin Stock, synthetic human Endothelin-1 (1-21), lyophilised, red cap, exact concentration after reconstitution see label</td>
<td>1 vial lyophilised</td>
</tr>
</tbody>
</table>

3) ADDITIONAL MATERIAL ADDED TO THE KIT
- 2 self-adhesive plastic films
- QC protocol
- Protocol sheet
- Instruction manual for use

4) MATERIAL AND EQUIPMENT REQUIRED BUT NOT SUPPLIED
- Precision pipettes calibrated to deliver 50µl, 200µl, 300µl, 500µl and disposable tips
- ELISA reader for absorbance at 450 nm (reference 630 nm)
- Graph paper or software for the calculation of results
- Distilled or deionised water

5) REAGENTS AND SAMPLE PREPARATION

All reagents of the Endothelin (1-21) ELISA Assay Kit are stable at 4°C (2-8°C) until expiry date stated on the label of each reagent.
Sample preparation:
Serum, EDTA plasma, urine, saliva and cell culture supernatants are suitable for use in this assay. Don’t change sample type during studies. freshly collected EDTA plasma or serum is put on ice immediately and centrifuged within one day. samples should be stored at -25°C or lower, 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 further information on sample stability please visit our www.bmgrp.com technical file or contact our customer service by e-mail export@bmgrp.com or by phone +43/ 1/ 29107-45.

Reconstitution/Handling:
STD (Standards, white caps) in 0.5ml distilled water, at room temperature (18-26°C) for 30 min, shake well. Reconstituted standards are stable at -25°C or lower 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 min, shake well. Reconstituted controls are stable at -25°C or lower until expiry date stated on label. Avoid repeated 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. Exact concentration after reconstitution see label, eg. 10 fmol/ml. Reconstituted ET stock is stable at -25°C or lower until expiry date stated on the label. Avoid repeated freeze-thaw cycles.

Measurement of Cell culture samples:
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.

Measurement of saliva samples:
For sample collection for the Endothelin (1-21) ELISA Assay Kit 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 -25°C or lower.
• Use the clear saliva according to the assay protocol.
6) **PRINCIPLE OF THE ASSAY**

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7) **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 aluminum bag, take 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/Sample/Control) in duplicate into respective well, except blank.
2. Add 200 µl AB (Detection antibody) into each well, except blank, swirl gently.
3. **Cover tightly and incubate overnight (16-24 hours) at room temperature (18-26°C).**
4. 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.
5. Add 200 µl CONJ (Conjugate) into each well.
6. **Cover tightly and incubate 1 hour at room temperature (18-26°C).**
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) into each well.
9. **Incubate for 30 minutes at room temperature (18-26°C) in the dark.**
10. Add 50 µl STOP (Stop solution) into each well, shake well.
11. Measure absorbance immediately at 450 nm with reference 630 nm, if available.

8) **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 Endothelin (1-21) ELISA Assay Kit has been evaluated using a 4PL algorithm. Different curve fitting methods need to be evaluated by the user. Respective dilution factors have to be considered.

**Example typical STD-curve:**

![Example typical STD-curve](image)

The quality control protocol supplied with the kit shows the results of the final release QC for each kit. Data for optical density 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 optical density of 1.50 or higher is obtained for the standard with the highest concentration.
9) ASSAY CHARACTERISTICS

Values from apparently healthy individuals: A panel of 70 blood donors had a median of 0.26 fmol/ml. Each laboratory should establish its own reference data.

Standard range: 0-10 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:

<table>
<thead>
<tr>
<th>Complement</th>
<th>Cross Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>ET(1-21)</td>
<td>100%</td>
</tr>
<tr>
<td>ET2 (1-21)</td>
<td>100%</td>
</tr>
<tr>
<td>ET3 (1-21)</td>
<td>&lt;5%</td>
</tr>
<tr>
<td>ET(1-38)</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>Big Endothelin (22-38)</td>
<td>&lt;1%</td>
</tr>
</tbody>
</table>

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.

For further information on assay characteristics please visit our website www.bmgrp.com technical file or contact our customer service by e-mail export@bmgrp.com or by phone +43/ 1/ 29107-45.

10) PRECISION

Intra-Assay: 2 samples of known concentrations were tested 18 times
Inter-Assay: 2 samples of known concentrations were tested in different assays 24 times

<table>
<thead>
<tr>
<th></th>
<th>Intra-Assay (n=18)</th>
<th>Inter-Assay (n=24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (fmol/ml)</td>
<td>2.02</td>
<td>3.8</td>
</tr>
<tr>
<td>SD</td>
<td>0.08</td>
<td>0.2</td>
</tr>
<tr>
<td>CV%</td>
<td>4%</td>
<td>6 %</td>
</tr>
</tbody>
</table>

11) 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 of the Endothelin (1-21) ELISA Assay Kit.
- 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.

12) 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.
- 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!

13) 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
SYMBOLS

Expiry date / Verfallsdatum / Date de péremption / Data di scadenza / Fecha de caducidad / Data de validade / Uiterste gebruiksdatum / Udløbsdato / Utgångsdatum / Termin Ważności / Lejárati idő / Doba exspiracie / Doba exspirace

Consider instructions for use / Bitte Gebrauchsanweisung beachten / Consultez la notice d'utilisation / Consultare le istruzioni per l'uso / Consulte las instrucciones de utilización / Consulte as instruções de utilização / Raadpleeg de gebruiksaanwijzing / Se brugsanvisningerne / Läs anvisningarna / Proszę przeczytać instrukcję wykonania / Vegyük figyelembe a használati utasításban / Postupujte podľa pokynov na použitie / Postupujte dle návodu / In vitro Diagnostic Medical Device (for in Vitro Diagnostic Use) / In vitro Diagnostikum (zur In-vitro-Diagnostik) / Dispositif médical de diagnostic in vitro (Pour usage diagnostique in vitro) / Dispositivo medico per diagnostica in vitro (per uso diagnostico in vitro) / Dispositivo médico de diagnóstico in vitro (para uso diagnóstico in vitro) / Dispositivo médico para diagnóstico in vitro (Para utilização de diagnóstico “in vitro”) / Medisch hulpmiddel voor diagnostiek in vitro (Voor diagnostisch gebruik in vitro) / Medicinsk udstyr til in-vitro-diagnostik (Udelukkende til in-vitro-diagnostisk anvendelse) / Medicinteknisk produkt avsedd för in-vitro-diagnostik (För in-vitro-diagnostisk bruk) / Wyrób medyczny do Diagnostyki In Vitro / In vitro orvosdiagnosztikai termék / In vitro diagnosticky zdravotnický materiál (určené pre diagnostiku „in vitro“) / In vitro diagnostický zdravotnický materiál (určeno pro diagnostiku „in vitro“)

Lot-Batch Number / Charge-Chargenummer / Lot-Code du lot / Lotto-Numero di lotto / Lote-Código de lote / Lote-Código do lote / Lot-Partijnummer / Lot-Batchcode / Lot-Satskod / Numer serii / Lot-Batch szám / Číslo šarže / Číslo šarže

Manufactured by / Hergestellt von / Fabriqué par / Prodotto da / Fabricado por / Fabricado por / Vervaardigd door / Fabrikation af / Tillverkad av / Wyprodukowane pr / Gyártotta / Vyrobené / Vyrobeno

Catalogue Number / Bestellnummer / Numéro de référence / Numero di riferimento / Número de referencia / Referentienummer / Referencenummer / Katalognummer / Numer katalogowy / Katalógusszám / Katalógové číslo / Katalogové číslo

Store at between / Lagerung bei zwischen / Conserver à entre / Conservare a tra / Conservar a temp. entre / Armazen e entre / Bewaar bij tussen / Opbevares mellem / Förvaras vid / Przechowywać w / Tároljuk ...... között / Skladujte v rozsahu / Skladujte v rozmezí

Contains sufficient for x tests / Inhalt ausreichend für x Teste / Contient suffisant pour x tests / Contenido suficiente para x pruebas / Contém suficiente para x testes / Bevat voldoende voor x bepalingen / Indeholder tilstrækkeligt til x prøver / Innehållet räcker till x analyser / Zawartość na x testów / Tartalma X teszt elvégzésére elegendő / Obsahuje materiál pre x testov / Obstahuje materiál pro x testů
BI-20052 ENDOTHELIN (1-21)
ASSAY PROTOCOL AND CHECKLIST

PREPARATION OF REAGENTS:

- Bring all reagents to room temperature (18-26°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 alubag and mark positions on the protocol sheet.

TEST PROCEDURE:

- Step 1) Add 50 µl STD/SAMPLE/CTRL (standard/sample/control) into all wells, except blank.
- Step 2) Add 200 µl AB (Detection antibody) into each well, except blank, swirl gently.
- Step 3) Cover tightly and incubate over night (16-24 hours) at room temperature (18-26°C).
- 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 (Conjugate) into each well.
- Step 6) Cover tightly and incubate at for 1 hour room temperature (18-26°C).
- 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) into each well.
- Step 9) Incubate for 30 minutes at room temperature (18-26°C) in the dark.
- Step 10) Add 50 µl STOP (Stop solution) into each well.
- Step 11) Read Optical Density at 450 nm with reference 630 nm, if available.
Warranty Information
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For further information about this kit, its application or the procedures in this kit insert, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.

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