Big ENDOTHELIN-1 ELISA Assay Kit

ENZYME IMMUNOASSAY FOR THE QUANTITATIVE DETERMINATION OF HUMAN BIG ENDOTHELIN-1 IN SERUM, CITRATE PLASMA, EDTA PLASMA OR HEPARIN PLASMA
CAT. NO. BI-20082H. 12 X 8 TESTS

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES
Additional information on our products is available on our website.

www.bmgrp.com
1) 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 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

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 sheep anti human Big Endothelin-1 antibody coated microtiter 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>Monoclonal mouse anti human Big Endothelin-1 antibody, biotin labelled, red dye, green cap, ready to use</td>
<td>1 x 18 ml</td>
</tr>
<tr>
<td>STD</td>
<td>Standards human sera, synthetic human Big Endothelin-1 (0, 0.10, 0.20, 0.40, 1, 3 pmol/l), lyophilised, white caps</td>
<td>6 vials</td>
</tr>
<tr>
<td>CTRL</td>
<td>Control human serum, synthetic human Big Endothelin-1, lyophilised, yellow cap, exact concentration after reconstitution see label</td>
<td>1 vial</td>
</tr>
<tr>
<td>CONJ</td>
<td>Conjugate, (streptavidin-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>
</tbody>
</table>

3) ADDITIONAL MATERIAL ADDED TO THE KIT

- 2 self-adhesive plastic film
- Quality control protocol
- Protocol sheet
- Instruction manual for use

4) MATERIAL AND EQUIPMENT REQUIRED BUT NOT SUPPLIED

- Precision pipettes calibrated to deliver 50 µl, 150 µl, 200 µl, 300 µl, 500 µl and disposable tips
- Distilled or deionised 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 450 nm (reference 630 nm)
- Graph paper or software for calculation of results
5) REAGENTS AND SAMPLE PREPARATION

All reagents of the Big Endothelin-1 ELISA Assay 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 chapter 9). 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 STD1 or BigET negative human serum. We recommend duplicates for all values.

For further information on sample stability 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.

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 deionised water into each vial. Leave at room temperature (18-26°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 deionised water to the vial. Leave at room temperature (18-26°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.

6) PRINCIPLE OF THE ASSAY

7) ASSAY PROTOCOL

All reagents and samples must be at room temperature (18-26°C) before use in the Big Endothelin-1 ELISA Assay Kit.

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

Take microtiter strips out of the aluminium bag, take a minimum of one well as Blank. Store unused strips with desiccant at 2-8°C in the aluminium 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 (18-26°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 CONJ (streptavidin-HRPO, amber cap) into each well.

6. **Cover tightly and incubate 1 hour at room temperature (18-26°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 (18-26°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.

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**8) 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 4PL 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 405nm (correction wavelength 630 nm).

**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 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).

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**9) ASSAY CHARACTERISTICS**

<table>
<thead>
<tr>
<th>Values from apparently healthy individuals (n=41):</th>
<th>Serum: Median: 0.09 pmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard range:</td>
<td>0-3 pmol/l</td>
</tr>
<tr>
<td>Conversion factor pg/ml to pmol/l:</td>
<td>1 pg/ml = 0.2335 pmol/l (MW: 4.283 kDa)</td>
</tr>
</tbody>
</table>

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Sample volume: 50 µl human serum or plasma (Citrate, EDTA or Heparin)
Detection limit: (0 pmol/l + 3 SD): 0.02 pmol/l
Incubation time: 4 h / 1 h / 30 min

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 5 times in 1 assay.
Inter-Assay: 2 samples of known concentrations were tested 10 times within 3 assays each by a different operator.

<table>
<thead>
<tr>
<th>Intra-Assay (n=5)</th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Inter-Assay (n=10)</th>
<th>Sample 1</th>
<th>Sample 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (pmol/l)</td>
<td>0.20</td>
<td>1.00</td>
<td>Mean (pmol/l)</td>
<td>0.20</td>
<td>1.00</td>
</tr>
<tr>
<td>SD (pmol/l)</td>
<td>0.003</td>
<td>0.048</td>
<td>SD (pmol/l)</td>
<td>0.009</td>
<td>0.041</td>
</tr>
<tr>
<td>CV%</td>
<td>2%</td>
<td>5%</td>
<td>CV%</td>
<td>4%</td>
<td>4%</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.
- 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.

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. Avoid contact with skin and mucous. Irritations are possible – flush with water if contact occurs!

13) LITERATURE
SYMBOLES

Expiry date / Verfallsdatum / Date de péremption / Fecha de caducidad / Data di scadenza / Data de validade / Uiterste gebruiksdatum / Udløbsdato / Utgångsdatum / Termin Ważności / Lejárati idő / Doba expirácie / 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 brugsanvisning / Läs anvisningarna före användning / Proszę przeczytać instrukcję wykonania / Vegyük figyelembe a használati utasításban / Postupujte podľa pokynov na použitie / Postupujte dle návodu k použití

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 para diagnóstico in vitro (Para utilización 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) / Medicinets produkt avsedd för in vitro-diagnostik (För in vitro-diagnostiskt bruk) / Wyrób medyczny do Diagnostyki In Vitro / In vitro orvosdiagnosztikai termék / In vitro diagnostický zdravotnický materiál (určené pre diagnostiku „in vitro“) / In vitro diagnostický zdravotnický materiál (určené pro diagnostiku „in vitro“)

Lot-Batch Number / Charge-Chargennummer / Lot-Code du lot / Lotto-Número di lotto / Lote-Código de lote / Lote-Código do lote / Lot-Partijnummer / Lot-Batchkode / Lot-Satskod / Numer serií / 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 / Número de referencia / Referentienummer / Referencenummer / Katalognummer / Numer katalogowy / Katalógussszám / Katalógové číslo / Katalogové číslo

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

Contains sufficient for x tests / Inhalt ausreichend für x Teste / Contient suffisant pour x tests / Contenuto sufficiente per x test / Contiene sufiiciente para x pruebas / Contêm 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 / Obsahuje materiál pro x testů
BI-20082H big ENDOTHELIN-1
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 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 (18-26°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.
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- Step 8) Add 200 µl SUB (substrate, blue cap) 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, white cap) into each well.
- Step 11) Read Optical Density at 450 nm with reference 630 nm, if available.
Warranty Information
Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

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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.

Manufactured by:
Biomedica Medizinprodukte GmbH & Co KG
A-1210 Wien, Divischgasse 4
Tel.+43/1/291 0745 • Fax +43/1/291 0785
www bmgrp.com • export@bmgrp.com

distributed in the US/Canada by:

EAGLE BIOSCIENCES, INC.
20A NW Blvd, Suite 112 Nashua, NH 03063
Phone: 617-419-2019 FAX: 617-419-1110
www.EagleBio.com • info@eaglebio.com