ENDOSTATIN ELISA Assay Kit

ENZYME IMMUNOASSAY FOR THE QUANTITATIVE DETERMINATION OF
ENDOSTATIN IN SERUM, CITRATE PLASMA, EDTA PLASMA, AND HEPARIN PLASMA
CAT. NO. BI-20742 . 12 X 8 TESTS

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

rev.no. 121218

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CONTENT / INHALT

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1) INTRODUCTION

Endostatin, a 20-kDa C-terminal proteolytic fragment of collagen XVIII, is an endogenous angiogenesis inhibitor localized in the vascular basement membrane in various organs (http://www.uniprot.org/uniprot/P39060). The biological functions of the endostatin-network involve SPARC, thrombospondin-1, glycosaminoglycans, collagens, and integrins.

Endostatin is expressed during the progression of renal fibrosis in tubular cells of injured tissue. In renal micro-vascular disease, observed in late stages of patients with chronic kidney disease, increased endostatin levels are possibly the consequence of enhanced extracellular matrix degradation. Thus endostatin may become an important marker for progressive microvascular renal disease in patients with chronic kidney disease. Endostatin levels in blood are also likely to increase in patients with other microvascular tissue injuries, including atherosclerosis, myocardial- and brain ischemia. In ischemic stroke patients, high endostatin plasma levels predict a worse long-term clinical outcome.

Areas of interest:

- Micro-vascular injury
- Chronic kidney disease
- Atherosclerosis
- Ischemia

2) CONTENT OF THE KIT

CONT	KIT COMPONENTS	QUANTITY
PLATE	Goat polyclonal anti endostatin antibody, pre-coated microtiter strips in a stripholder, packed in an aluminium bag with desiccant	12 x 8 tests
WASHBUF	Wash buffer concentrate 20x, natural cap	1 x 50 ml
AB	Rabbit polyclonal anti endostatin antibody – biotin labelled, natural cap, green dye, ready to use	1 x 7 ml
STD	Standards 1-6, (0; 5; 10; 20; 40, 80 nmol/l), white caps	6 x 150 µl
CTRL	Control, yellow cap, lyophilized (exact concentration on the label)	1 vial
ASYBUF	Assay Buffer, natural cap, ready to use	1 x 120 ml
CONJ	Conjugate, (streptavidin-HRPO), amber bottle, amber cap, ready to use	1 x 22 ml
SUB	Substrate (TMB solution), amber bottle, blue cap, ready to use	1 x 22 ml
STOP	Stop solution, white cap, ready to use	1 x 7 ml

3) ADDITIONAL MATERIAL IN THE KIT

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

4) MATERIAL AND EQUIPMENT REQUIRED BUT NOT SUPPLIED

- Precision pipettes calibrated to deliver 20 µl, 50 µl, 100 µl, 200 µl, 300 µl, 1000 µl and disposable tips
- Distilled or deionised water
- Plate washer is recommended for washing, alternative multichannel pipette or manifold dispenser
- ELISA reader capable of measuring absorbance at 450 nm (with correction wavelength at 630 nm)

5) REAGENTS AND SAMPLE PREPARATION

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

Sample preparation:

Collect venous blood samples by using standardized blood collection tubes for serum or plasma. We recommend the separation of plasma or serum by centrifugation as soon as possible (e.g. 20 min at 2000 x g, preferably at 4°C (2-8°C)). If this is not possible store the samples at 4°C (2-8°C) prior to centrifugation (up to one day).
The acquired plasma or serum samples should be measured as soon as possible. For longer storage aliquot samples and store at -25°C or lower. Samples are at least stable for 4 freeze-thaw cycles. Lipemic or haemolysed samples may give erroneous results. Samples should be mixed well before assaying.

Samples must be diluted 1+100 with **assay buffer (ASYBUF), eg. 10 µl sample + 1000 µl ASYBUF.**
Diluted samples are stable at 4°C (2-8°C) overnight. Thus dilutions can be prepared one day before analysis.

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

Reconstitution/Handling:

WASHBUF (Wash buffer): Salt precipitate in the concentrated wash buffer is normal. Dissolve any precipitate by mixing gently at room temperature then dilute the concentrate 1:20 with distilled/DI water (e.g. 50 ml WASHBUF + 950 ml distilled water) prior to using in the assay. Diluted wash buffer is stable at 4°C (2-8°C) for one month. Only use diluted WASHBUF (Wash buffer) for optimum assay performance.

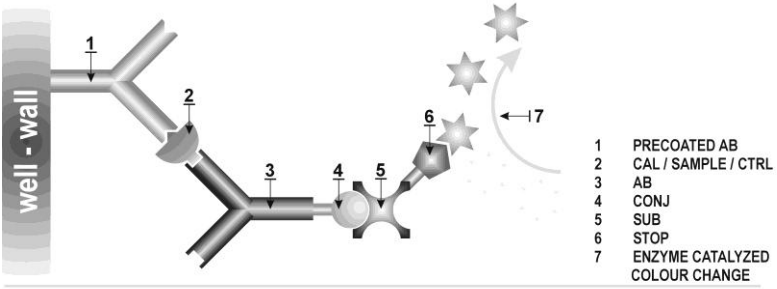
CTRL (Control): Pipette 150 µl of distilled or deionised water into the vial. Leave at room temperature (18-26°C) for 20 min. Swirl gently. The exact concentration is printed on the label. The reconstituted CTRL is stable at -25°C or lower until expiry date. Reconstituted CTRL is at least stable for 3 freeze-thaw cycles.

Control (CTRL) must be diluted 1+100 with **assay buffer (ASYBUF), eg. 10 µl CTRL + 1000 µl ASYBUF.**
Diluted control (CTRL) is stable at 4°C (2-8°C) overnight. The dilution can be prepared one day before analysis.

STD (Standards): Standards are stable at 4°C (2-8°C) until expiry date.

Standards (STD) must be diluted 1+100 with **assay buffer (ASYBUF), eg. 10 µl STD + 1000 µl ASYBUF.**
Diluted standards (STD) are stable at 4°C (2-8°C) overnight. Thus dilutions can be prepared one day before analysis.

6) PRINCIPLE OF THE ASSAY



7) ASSAY PROTOCOL

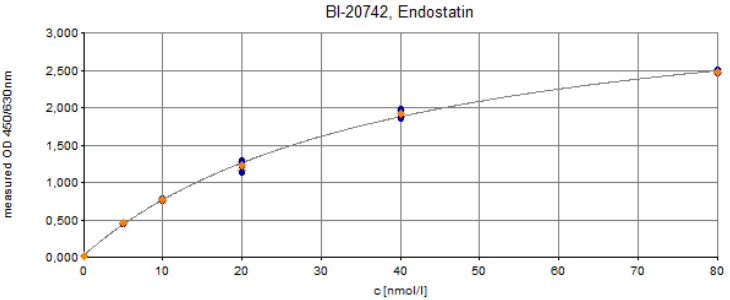
All reagents and samples must be at room temperature (18-26°C) before use in the Endostatin ELISA Assay Kit.
Mark position for BLANK/STD/SAMPLE/CTRL (Blank/Standard/Sample/Control) on the protocol sheet.
Take microtiter strips out of the aluminium bag, take a minimum of one well as Blank. Store unused strips with desiccant at 4°C (2-8°C) in the aluminium bag. Strips are stable until expiry date stated on the label

1) Pipette 100 µl ASYBUF (Assay Buffer, natural cap) into each well. Pipette additional 100 µl into well marked as blank.
2) Add 20 µl of 1+100 diluted STD/SAMPLE/CTRL (Standard/Sample/Control) in duplicate into respective well, except blank.
3) Add 50 µl AB (Antibody) into each well, swirl gently.
4) Cover tightly and incubate 3 hours at room temperature (18-26°C).
5) Aspirate and wash wells 5x with 300 µl diluted WASHBUF (Wash buffer). After final wash, remove remaining WASHBUF by strongly tapping plate against paper towel.
6) Add 200 µl CONJ (Conjugate, amber cap) into each well.
7) Cover tightly and incubate for 1 hour at room temperature (18-26°C).
8) Aspirate and wash wells 5x with 300 µl diluted WASHBUF (Wash buffer). After final wash, remove remaining WASHBUF by strongly tapping plate against paper towel.
9) Add 200 µl SUB (Substrate, blue cap) into each well.
10) Incubate for 30 min at room temperature (18-26°C) in the dark.
11) Add 50 µl STOP (Stop solution, white cap) into each well.
12) Measure absorbance immediately at 450 nm with reference 630 nm, if available.

8) CALCULATION OF RESULTS

Read the optical density (OD) of all wells with 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 commercially available software or graph paper. Obtain sample concentration from this standard curve. The assay was evaluated with 4PL algorithm. Different curve fitting methods need to be evaluated by the user. Due to the fact that STDs and samples are diluted 1+100 - no dilution factors have to be considered when calculating the results. Eventual additional dilution steps for samples have to be considered.

Example typical STD-curve:



The quality control (QC) protocol supplied with the Endostatin ELISA Assay 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 1.50 or more is obtained for the STD with the highest concentration and the value of the CTRL is in range (target range see label).

9) ASSAY CHARACTERISTICS

Values from apparently healthy individuals	Median: 5.1 nmol/l (n=59, Serum) Median: 4.7 nmol/l (n=30, Citrate plasma) Each laboratory should establish its own reference range for the samples under investigation. Do not change sample type during study.
Standard range	0-80 nmol/l
Conversion factor ng/ml to nmol/l:	1 ng/ml = 0.05 nmol/l (MW: 20 kDa)

Sample volume:	20 µl of 1+100 diluted human serum or plasma (Citrate, EDTA or Heparin)
Detection limit:	(0 nmol/l + 3 SD): 0.2 nmol/l
Incubation time:	3 h / 1 h / 30 min

For further information on assay characteristics of the Endostatin ELISA Assay Kit please visit our website www.bmgrp.com 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 within one kit lot by one operator.
Inter-assay: 2 samples of known concentrations were tested 16 times within 2 different kit lots and by 3 different operators.

Intra-assay (n=5)	Sample 1	Sample 2		Inter-assay (n=16)	Sample 1	Sample 2
Mean (nmol/l)	5.2	41.6		Mean (nmol/l)	5.0	40.5
SD (nmol/l)	0.09	2.36		SD (nmol/l)	0.21	2.16
CV (%)	2	6		CV (%)	4	5

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 Endostatin ELISA Assay Kit 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 the Endostatin ELISA Assay Kit 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. Avoid contact with skin and mucous membrane. 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.
- Wear gloves, glasses and lab jacket while performing this assay.
- Sulfuric acid is irritating to the eyes and skin. Avoid contact with skin and mucous. Irritations are possible. – Flush with water if contact occurs!!

13) LITERATURE

1. Elevated plasma levels of endostatin are associated with chronic kidney disease. *Chen et al., 2012, Am J Nephrol 35(4): 335-340.*
2. Early-onset coronary artery disease after pediatric kidney transplantation: implicating the angiogenesis inhibitor, endostatin. *Igbal CW et al., 2011, Am Surg 77(6): 731-735.*
3. A defective angiogenesis in chronic kidney disease. *Futrakul N et al., 2008, Ren Fail 30(2): 215-217.*
4. Excretion of anti-angiogenic proteins in patients with chronic allograft dysfunction. *Moskowitz-Kassai E et al., 2012, Nephrol Dial Transplant 27(2): 494-497.*
5. Endostatin and angiostatin are increased in diabetic patients with coronary artery disease and associated with impaired coronary collateral formation. *Sodha NR et al., 2009, Am J Physiol Heart Circ Physiol, 296: H428-H434.*
6. A large screening of angiogenesis biomarkers and their association with neurological outcome after ischemic stroke. *Navarro-Sobrino M et al., 2011, Atherosclerosis, 216(1): 205-211.*

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 exspirácie / 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 brugsanvisningen / Läs anvisningarna före användning / Proszę przeczytać instrukcję wykonania / Vegyük figyelembe a használati utasításban foglaltakat / 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 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-diagnostiskt bruk) / Wyrób medyczny do Diagnostyki In Vitro / In vitro orvosdiagnosztikai termék / In vitro diagnostický zdravotnícky materiál (určené pre diagnostiku „in vitro“) / In vitro diagnostický zdravotnicky materiál (určeno pro diagnostiku „in vitro“)

Lot-Batch Number / Charge-Chargennummer / Lot-Code du lot / Lotto-Numero di lotto / Lote-Código de lote / Lote-Código do lote / Lot-Partijnummer / Lot-Batchkode / 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

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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 / Skladujte v rozsahu / Skladujte v rozmezí

Contains sufficient for x tests / Inhalt ausreichend für x Tests / Contient suffisant pour x tests / Contenuto sufficiente per x test / Contiene 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 / Obsahuje materiál pro x testů

BI-20742 ENDOSTATIN

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 aluminium bag and mark positions on the protocol sheet.

TEST PROCEDURE:

- ❑ Step 1) Pipette 100 µl ASYBUF (natural cap) into respective well. Pipette additional 100 µl into well marked as blank.
- ❑ Step 2) Add 20 µl of **1+100 diluted STD/SAMPLE/CTRL** (Standard/Sample/Control) in duplicate into respective well, except blank.
- ❑ Step 3) Add 50 µl AB (biotinylated anti Endostatin antibody, natural cap, green dye) into each well, swirl gently.
- ❑ **Step 4) Cover tightly and incubate 3 hours at room temperature (18-26°C).**
- ❑ Step 5) Aspirate and wash wells 5x with 300 µl diluted WASHBUF (Wash buffer). After final wash, remove remaining WASHBUF by strongly tapping plate against paper towel.
- ❑ Step 6) Add 200 µl CONJ (Conjugate, amber cap) into each well.
- ❑ **Step 7) Cover tightly and incubate for 1 hour at room temperature (18-26°C).**
- ❑ Step 8) Aspirate and wash wells 5x with 300 µl diluted WASHBUF (Wash buffer). After final wash, remove remaining WASHBUF by strongly tapping plate against paper towel.
- ❑ Step 9) Add 200 µl SUB (Substrate, blue cap) into each well.
- ❑ **Step 10) Incubate for 30 min at room temperature (18-26°C) in the dark.**
- ❑ Step 11) Add 50 µl STOP (Stop solution, white cap) into each well.
- ❑ Step 12) Read Optical Density immediately at 450 nm with reference 630 nm, if available.

Warranty Information

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