NT-proCNP
2nd generation

ENZYME IMMUNOASSAY FOR THE QUANTITATIVE DETERMINATION OF NT-proCNP IN SERUM, EDTA PLASMA, CITRATE PLASMA OR HEPARIN PLASMA CAT. NO. BI-20812 . 12 X 8 TESTS

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

rev.no. 171213
Detailed information on the assay characteristics including the validation data can be found on our website.

www.bmgrp.com
1) INTRODUCTION

C-type natriuretic peptide (CNP) is a paracrine growth factor widely expressed in tissues, including the vascular endothelium, where it is considered to provide vasoprotective functions. In endothelial cells and macrophages it is secreted in response to several stimuli, including inflammatory mediators. CNP is rapidly degraded in tissues and negligible quantities enter the circulation. However, the N-terminal portion of the pro-hormone is not degraded at source and circulates in significantly higher concentrations than CNP. Therefore NT-proCNP is a valuable biomarker to determine CNP synthesis in tissues. CNP plays a critical role in linear growth. It is produced in the growth plate and signals through a paracrine mechanism. Recent studies have shown that the plasma concentrations of NT-proCNP correlate with linear growth velocity in all phases of skeletal growth and increase during rhGH therapy (1). Furthermore, serum NT-proCNP levels increased after initiation of GH treatment in patients with achondroplasia/hypochondroplasia (2). Women with pregnancy complications, such as diminished fetal growth and pre-eclampsia show significantly increased NT-proCNP levels early in gestation (3, 4). NT-proCNP concentration at hospital admission has sufficient sensitivity and specificity to differentiate naturally occurring sepsis from non-septic systemic inflammatory response syndrome (SIRS) (5, 6). Recently, Prickett and colleagues demonstrated in a cohort of over 2000 individuals, that in contrast to the close association of NT-proBNP with cardiac function, and predictive value for outcome after myocardial infarction, plasma NT-proCNP is highly correlated with renal function and is an independent predictor of mortality and cardiac readmission in individuals with unstable angina (7).

Areas of Interest
- Vascular disease
- Growth
- Skeletal development
- Angiogenesis
- Sepsis

2) CONTENT 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 NT-proCNP antibody precoated 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>ASYBUF</td>
<td>Assay buffer, red cap, ready to use</td>
<td>1 x 8 ml</td>
</tr>
<tr>
<td>STD</td>
<td>Standards, (0; 4; 8; 16; 32; 64; 128 pmol/l), white caps, lyophilised</td>
<td>7 vials</td>
</tr>
<tr>
<td>CTRL</td>
<td>Controls A + B, yellow caps, lyophilised, exact concentrations see labels</td>
<td>2 vials</td>
</tr>
<tr>
<td>CONJ</td>
<td>Conjugate (sheep anti NT-proCNP-HRPO), amber cap, ready to use</td>
<td>1 x 7 ml</td>
</tr>
<tr>
<td>SUB</td>
<td>Substrate (TMB solution), blue cap, ready to use</td>
<td>1 x 13 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
- 1 self-adhesive plastic film
- QC protocol
- Protocol sheet
- Instruction manual

4) EQUIPMENT REQUIRED BUT NOT SUPPLIED
- Precision pipettes calibrated to deliver 20 µl, 50 µl, 100 µl, and 300 µl and disposable tips
- ELISA reader for absorbance at 450 nm (reference 630 nm)
- Graph paper or software for calculation of results
- Distilled or deionised water

5) REAGENTS AND SAMPLE PREPARATION

All reagents of the 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. Perform serum/plasma separation by centrifugation according to supplier’s instructions of the blood collection devices as soon as possible. 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. All samples should undergo only 4 freeze-thaw cycles. Lipemic or haemolysed samples may give erroneous results. Samples should be mixed well before assaying. We recommend duplicates for all values. Samples with values above highest STD can be diluted 1+1 or 1+3 with ASYBUF (Assay buffer).

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

Reconstitute as follows:
WASHBUF (Wash buffer): Dilute the concentrate 1:20 (1+19), e.g. 50 ml WASHBUF + 950 ml distilled water. Crystals in the buffer concentrate will dissolve at room temperature. Undiluted WASHBUF is stable at +4°C (2-8°C) until expiry date stated on label. Diluted WASHBUF is stable at +4°C (2-8°C) for one month. Use only diluted WASHBUF to perform the assay.

STD (Standards) + CTRL (Controls): Pipette 300 µl of distilled or deionised water into each vial. Leave at room temperature (18-26°C) for 15 min. Vortex gently. The exact concentration is printed on the label. Reconstituted STDs and CTRLs are stable at -25°C or lower until expiry date stated on the label. STDs and CTRLs are stable for 3 freeze-thaw cycles.

6) PRINCIPLE OF THE ASSAY
This kit is a sandwich enzyme immunoassay for the determination of NT-proCNP in human samples. In a first step, assay buffer and sample are pipetted into the wells of the microtiter strips, which are pre-coated with polyclonal sheep anti NT-proCNP antibody, for a short incubation. Without the need of a washing step, conjugate (sheep anti human NT-proCNP-HRPO) is added into the wells. NT-proCNP present in the sample binds to the pre-coated antibody in the well and forms a sandwich with the detection antibody. In the washing step all non-specific unbound material is removed. After washing the substrate (TMB Tetramethylbenzidine) is pipetted into the wells. The enzyme catalysed colour change of the substrate is directly proportional to the amount of NT-proCNP present in the sample. This colour change is detectable with a standard microtiter plate ELISA reader.
**7) ASSAY PROTOCOL**

All reagents and samples have to be brought to room temperature (18-26°C) before they can be used in the assay. Mark position for STD/SAMPLE/CTRL (Standard/Sample/Control) on the protocol sheet.

Take microtiter strips out of the aluminium bag. Unused strips can be stored with desiccant in the aluminium bag at +4°C (2-8°C) until the expiry date.

1. Pipette 50 µl ASYBUF (Assay buffer, red cap) into each well.
2. Add 20 µl STD/CTRL/SAMPLE (Standard/Control/Sample) in duplicate into respective wells, swirl gently.
3. **Cover tightly and incubate for 20 minutes at room temperature (18-26°C).**
4. Add 50 µl CONJ (Conjugate, amber cap) into each well, swirl gently.
5. **Cover tightly and incubate for 3 hours at room temperature (18-26°C) in the dark.**
6. Aspirate and wash wells 5x with 300 µl diluted WASHBUF (Wash buffer), remove remaining WASHBUF by hitting plate against paper towel after the final washing step.
7. Add 100 µl SUB (Substrate, blue cap) into each well, swirl gently.
8. **Incubate for 30 min at room temperature (18-26°C) in the dark.**
9. Add 50 µl STOP (Stop solution, white cap) into each well, swirl gently.
10. Measure absorbance immediately at 450 nm with reference 630 nm, if available.

For the measurement of NT-proCNP in human urine, cell culture supernatants and non-human samples please visit our website [www.bmgrp.com](http://www.bmgrp.com) (see Validation Data).

**8) CALCULATION OF RESULTS**

Read the optical density (OD) of all wells on a plate reader using 450 nm wavelength (correction wavelength 630 nm). 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. Respective dilution factors have to be considered.

**Typical STD-curve:**

![NT-proCNP BI-20612](image)

The quality control (QC) 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 1.50 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**

<table>
<thead>
<tr>
<th>Method:</th>
<th>Sandwich ELISA, HRP/TMB, 12x8-well strips</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample type:</td>
<td>Serum, EDTA plasma, heparin plasma, and citrate plasma Protocols available for urine, cell culture supernatant and non-human species</td>
</tr>
<tr>
<td>Standard range:</td>
<td>0 to 128 pmol/l (7 standards and 2 controls in a human serum matrix) Standard points: 0 / 4 / 8 / 16 / 32 / 64 / 128 pmol/l</td>
</tr>
<tr>
<td>Conversion factor:</td>
<td>1 pg/ml = 0.201 pmol/l (MW: 4.985 kD) 1 pmol/l = 4.985 pg/ml</td>
</tr>
<tr>
<td>Sample volume:</td>
<td>20 µl / well</td>
</tr>
<tr>
<td>Incubation time, temperature:</td>
<td>20 min / 3 h / 30 min, room temperature</td>
</tr>
<tr>
<td>Sensitivity:</td>
<td>LOD: (0 pmol/l + 3 SD): 0.7 pmol/l; LLOQ: 0.5 pmol/l</td>
</tr>
<tr>
<td>Specificity:</td>
<td>This assay recognizes endogenous and synthetic human NT-proCNP.</td>
</tr>
<tr>
<td>Precision:</td>
<td>Intra-assay (n=5) ≤ 6%, Inter-assay (n=8) ≤ 7%</td>
</tr>
<tr>
<td>Spike/Recovery (average recovery spiked with 64 pmol/l synthetic NT-proCNP):</td>
<td>Serum (n=6): 101% Heparin plasma (n=2): 93% EDTA plasma (n=6): 99% Citrate plasma (n=2): 100%</td>
</tr>
<tr>
<td>Dilution linearity of endogenous NT-proCNP:</td>
<td>Average % expected of dilution: 1+1 1+3 Serum (n=6) 99 98 EDTA plasma (n=6) 103 98 Heparin plasma (n=2) 100 100 Citrate plasma (n=2) 96 92</td>
</tr>
<tr>
<td>Values from apparently healthy individuals:</td>
<td>Median serum (n=32) = 14.5 pmol/l Median EDTA plasma (n=33) = 15 pmol/l Median heparin plasma (n=18) = 13.5 pmol/l Median citrate plasma (n=18) = 12 pmol/l Each laboratory should establish its own reference range for the samples under investigation. Do not change sample type during the study.</td>
</tr>
</tbody>
</table>

For further information on assay characteristics and antibody specificity please visit our website **www.bmgrp.com** (see Validation Data) or contact our customer service by e-mail **export@bmgrp.com** or by phone +43/ 1/ 29107-45.

10) **PRECISION**

Intra-assay: 2 samples were tested 5 times within 1 assay lot by 1 operator. Inter-assay: 2 samples were tested 8 times in 2 different kit lots by 2 different operators.

<table>
<thead>
<tr>
<th>Intra-assay (n=5)</th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Inter-assay (n=8)</th>
<th>Sample 1</th>
<th>Sample 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (pmol/l)</td>
<td>7.9</td>
<td>65.3</td>
<td>Mean (pmol/l)</td>
<td>8.2</td>
<td>64.1</td>
</tr>
<tr>
<td>SD (pmol/l)</td>
<td>0.47</td>
<td>1.25</td>
<td>SD (pmol/l)</td>
<td>0.54</td>
<td>1.42</td>
</tr>
<tr>
<td>CV (%)</td>
<td>6</td>
<td>2</td>
<td>CV (%)</td>
<td>7</td>
<td>2</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 colorless 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 against HIV-Ab, HCV-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. The stop solution contains sulfuric acid, contact can lead to irritations of eyes and skin. Flush with water after contact!

13) LITERATURE

8. The natriuretic peptides system in the pathophysiology of heart failure: from molecular basis to treatment. Volpe M et al., Clinical Science, 2016; 130:57-77.
SYMBOLS

Expiry date / Verfallsdatum / Date de péremption / Fecha de caducidad / Data de validade / Uiterste gebruiksdatum / Udløbsdato / Utgångsdatum / Termin Ważności / Lejáratid / Doba exspiracije / 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 / Vegyünk 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 diagnostic in vitro (per uso diagnostico 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-diagnostikk) / Medicinteknik produkt avsedd för in vitro-diagnostik (For in vitro-diagnostiskt 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 / Vyrobene / 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 / 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 / Contenido suficiente para x testes / Contém suficiente para x testes / Bevat voldoende voor x testen / Indeholder tilstrækkeligt til x prøver / Innehåller räcker till x analyser / Zawartość na x testów / Tartalma X teszt elvégzésére elegendő / Obsahuje materiál pre x testov / Obsahuje materiál pro x testů
BI-20812 NT-proCNP
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:
- 1) Pipette 50 µl ASYBUF (Assay Buffer) into each well.
- 2) Add 20 µl STD/ SAMPLE/ CTRL (standard/ sample/ control) in duplicates into respective wells, swirl gently.
- 3) Cover tightly and incubate for 20 min at room temperature (18-26°C).
- 4) Add 50 µl CONJ (Conjugate, amber cap) into each well, swirl gently.
- 5) Cover tightly and incubate for 3 hours at room temperature (18-26°C) in the dark.
- 6) Aspirate and wash wells five times with 300 µl diluted WASHBUF (Wash buffer). Remove remaining buffer by hitting plate against paper towel.
- 7) Add 100 µl SUB (Substrate) into each well, swirl gently.
- 8) Incubate for 30 minutes at room temperature (18-26°C), in the dark.
- 9) Add 50 µl STOP (Stop solution) into each well, swirl gently.
- 10) Read optical density at 450 nm with reference 630 nm, if available.

For the measurement of NT-proCNP in human urine, cell culture supernatants and non-human samples please visit our website www.bmgrp.com (see Validation Data).
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.

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