

NT-proCNP ELISA ASSAY KIT

ENZYME IMMUNOASSAY FOR THE QUANTITATIVE DETERMINATION OF HUMAN
NT-proCNP IN PLASMA AND SERUM
CAT. NO. BI-20872 12 X 8 TESTS

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

rev.no. 120627 (replacing 110331)
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**BIOMEDICA**

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1) INTRODUCTION

CNP belongs to a well characterised group of natriuretic peptides like atrial natriuretic (ANP) and brain natriuretic peptide (BNP), which play various important roles as regulatory mechanisms as well as bio-markers. They participate in the regulation of blood pressure and body fluid homeostasis, modify growth and development of cardiovascular tissues and bone. ANP and BNP are cardiac hormones, secreted by the atria and ventricles, respectively, in the normal adult heart. In contrast to ANP and BNP, very little CNP is produced by cardiac tissue, but is expressed primarily in the brain, pituitary gland, vascular endothelium, kidney and female reproductive tract.

All natriuretic peptides are produced as propeptides, which are subsequently cleaved into the biologically active, C-terminal hormone and the N-terminal fragment (NT-proANP 1-98, NT-proBNP 1-76 and NT-proCNP, which length is still under discussion). Since those N-terminal fragments are much more stable, and circulate in higher amounts than the active hormones, they are easier and more reliable to be measured in serum or plasma. So we developed a sandwich immunoassay for NT-proCNP using antibodies directed against amino acids 1-19 and 30-50. The physiological role of CNP is not only studied in cardiac disease, but also in bone developmental biology, generally in bone research, renal diseases, embryonic developmental research and vascular diseases.

Indications:

- vascular disease
- diabetes
- skeletal development
- sepsis

2) CONTENTS OF THE KIT

CONT	KIT COMPONENTS	QUANTITY
PLATE	Polyclonal sheep anti NT-proCNP antibody precoated microtiter strips in stripholder packed in aluminium bag with desiccant	12 x 8 tests
WASHBUF	Wash buffer concentrate 20x, natural cap	1 x 50 ml
ASYBUF	Assay buffer, red cap, ready to use	1 x 10 ml
STD	Standards, (0; 2.5; 5; 10; 20; 40 pmol/l), white caps, ready to use	6 x 500 µl
CTRL	Control, yellow cap, ready to use, exact concentration see label	1 x 500 µl
CONJ	Conjugate, (anti NT-proCNP-HRPO), amber cap, ready to use	1 x 22 ml
SUB	Substrate (TMB solution), blue cap, ready to use	1 x 22 ml
STOP	STOP solution, white cap, ready to use	1 x 7 ml

3) ADDITIONAL MATERIAL ADDED TO THE KIT

- 2 self-adhesive plastic film (aluminium foil)
- QC protocol
- Protocol sheet
- Instruction manual

4) EQUIPMENT REQUIRED BUT NOT SUPPLIED

- Precision pipettes calibrated to deliver 50 µl, 200 µl, 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 NT-proCNP ELISA Assay Kit are stable at +4°C (2-8°C) until expiry date stated on the label of each reagent.

Sample preparation:

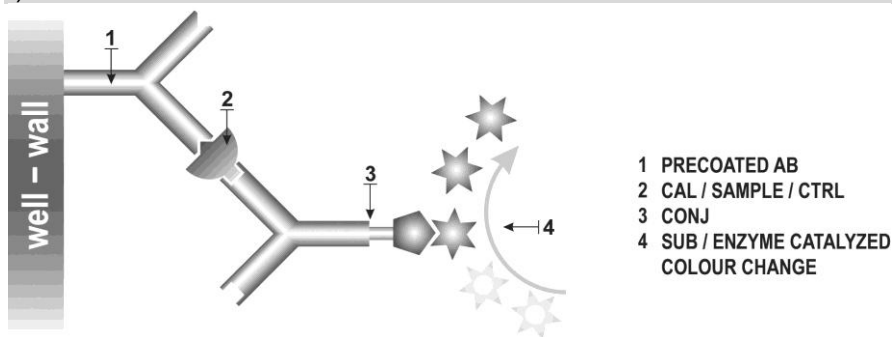
proCNP in freshly collected blood samples is stable for at least 2.5 hrs at RT. Nevertheless we recommend to perform plasma or serum separation by centrifugation as soon as possible (e.g. 20 min at 2000 x g, preferably at +4°C (2-8°C)). Aliquot the acquired plasma or serum samples and store them at -25°C or lower. Samples can be subjected to 5 freeze-thaw cycles without any loss of immune reactivity. Lipemic or hemolyzed samples may give erroneous results. Samples should be mixed well before assaying. We recommend duplicates for all values. If samples read higher than the highest STD we recommend to dilute them with ASYBUF (assay buffer) (e.g.: 1+1 and 1+3) and re-measure the samples.

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.

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. Buffer is stable at +4°C (2-8°C) until expiry date stated on label. Use only diluted WASHBUF (Wash buffer) to perform the assay.

6) PRINCIPLE OF THE ASSAY



7) ASSAY PROTOCOL

All reagents and samples have to be brought to room temperature (18-26°C) before they can be used in the NT-proCNP ELISA Assay Kit.

Mark position for BLANK/STD/SAMPLE/CTRL (Blank/Standard/Sample/Control) on the protocol sheet.

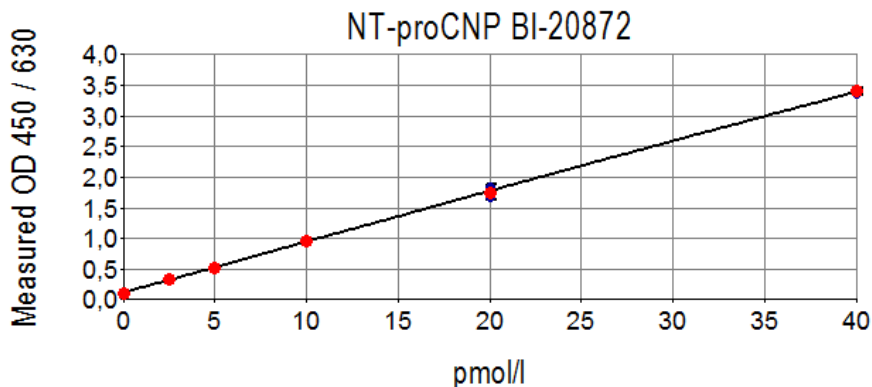
Take microtiter strips out of the aluminium bag, reserve a minimum of one well as blank. Unused strips can be stored with desiccant in the aluminium bag at +4°C (2-8°C) until the expiry date.

1. Add 50 µl STD/SAMPLE/CTRL (Standard/Sample/Control) in duplicate into respective wells, except blank.
2. Add 200 µl CONJ (anti NT-proCNP-HRPO) into each well, except blank, swirl gently.
3. **Cover tightly and incubate for 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 final washing step.
5. Add 200 µl SUB (Substrate) into each well.
6. **Incubate for 30 min at room temperature (18-26°C) in the dark.**
7. Add 50 µl STOP (Stop solution) into each well.
8. Measure absorbance immediately at 450 nm with reference 630 nm, if available.

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 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.

Example typical STD-curve:



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

Apparently healthy individuals and hospital panel:	Serum, apparently healthy: 2.7 pmol/l (median, n=42) Serum, hospital panel: 2.8 pmol/l (median, n=23) EDTA plasma, hospital panel: 3.0 pmol/l (median, n=25) Each laboratory should establish own normal values.
Standard range:	0 – 40 pmol/l
Conversion factor:	1pg/ml = 0.151 pmol/l (referring to recombinant proCNP 1-64)
Sample volume:	50 µl human serum or plasma
Detection Limit:	(0 pmol/l + 3 SD): 0.2 pmol/l
Incubation time:	4 hours / 30 min
Cross reactivity:	Not determined, homology to other species for the epitopes used in this assay: Epitope 1: rat (88%), sheep (94%), pig (94%), mouse (88%), bovine (88%) Epitope 2: rat (95%), sheep (90%), pig (95%), mouse (95%), bovine (90%)

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 were tested 5 times within 1 assay lot by one operator.

Inter-Assay: 2 samples were tested 30 times in 3 different lots by 4 different operators.

Intra-Assay (n=5)	Sample 1	Sample 2
Mean (pmol/l)	5.1	20.0
SD (pmol/l)	0.26	1.17
CV%	5%	6%

Inter-Assay (n=30)	Sample 1	Sample 2
Mean (pmol/l)	5.0	20.0
SD (pmol/l)	0.07	0.15
CV%	1%	1%

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 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 $\leq 0.1\%$ 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

1. "Identification of amino-terminal pro-C-type natriuretic peptide in human plasma"
T.C.R Prickett et al., Biochem. and Biophys. Res. Comm., 286, 513-517 (2001)
2. "Overexpression of CNP in chondrocytes rescues achondroplasia through a MAPK- dependent pathway"
Akihiro Yasoda et al., Nature Medicine, 10, 80-86 (Dez 2003)
3. "C-type natriuretic peptide in reproduction, pregnancy, and fetal development"
T. Walther et al. J. of Endo, 180, 17-22 (2004)

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 expirácie / Doba expirace



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 / Vegyűk 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 zdravotnícký materiál (určen pre diagnostiku „in vitro“) / In vitro diagnostický zdravotnícký 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



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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-20872 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:

- Step 1) Add 50 µl STD/ SAMPLE/ CTRL (standard/diluted sample/diluted control) into each well, except blank.
- Step 2) Add 200 µl CONJ (Conjugate) into each well, 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 diluted WASHBUF (Wash buffer) five times. Remove remaining buffer by hitting plate against paper towel.
- Step 5) Add 200 µl SUB (Substrate) into each well.
- Step 6) Incubate for 30 minutes at room temperature (18-26°C), in the dark.**
- Step 7) Add 50 µl STOP (Stop solution) into each well.
- Step 8) Read optical density at 450 nm with reference 630 nm, if available.

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