ELISA PRODUCT INFORMATION & MANUAL

CNP/NPPC

NBP2-62165

Enzyme-linked Immunosorbent Assay for quantitative detection of Human CNP/NPPC.

For research use only.

Not for diagnostic or therapeutic procedures.

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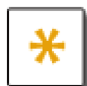
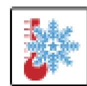


Please read entire booklet before proceeding with the assay.



Carefully note the handling and storage conditions of each kit component.

Please contact Novus Biologicals Technical Support if necessary.

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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 NTproCNP 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 colleages 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).

APPLICATION

Areas of Interest:

- Vascular Disease
- Growth
- Skeletal Development
- Angiogenesis
- Sepsis

MATERIALS SUPPLIED

Do not mix components from different kit lots or use reagents beyond the expiration date of the kit.

1. **Polyclonal sheep anti NT-proCNP antibody precoated microtiter strips, 12 x 8 tests**

   In stripholder packed in aluminum bag with desiccant
2. **Wash buffer concentrate 20X, natural cap, 50 mL**
3. **Assay buffer, red cap, 8 mL**

   Ready to use
4. **Lyophilised standards, white caps, 7 vials**

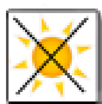
   0, 4, 8, 16, 32, 64, 128 pmol/L
5. **Lyophilised controls A + B, yellow caps, 2 vials**

   See labels for exact concentrations
6. **Sheep anti NT-proCNP-HRPO conjugate, amber cap, 7 mL**

   Ready to use
7. **Substrate (TMB solution), blue cap, 13 mL**

   Ready to use
8. **Stop solution, white cap, 7 mL**

   Ready to use



Protect substrate from prolonged exposure to light.

ADDITIONAL MATERIALS ADDED

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

ADDITIONAL MATERIALS AND EQUIPMENT NEEDED

- Precision pipettes calibrated to deliver 20 µL, 50 µL, 100 µL, 300 µL and disposable tips
- Distilled or deionized water
- ELISA reader for absorbance at 450nm (reference 630nm)
- Graph paper or software for calculation of results

Stop solution is caustic. Keep tightly capped.

STORAGE

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

REAGENTS AND 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 hemolyzed 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 assay buffer.

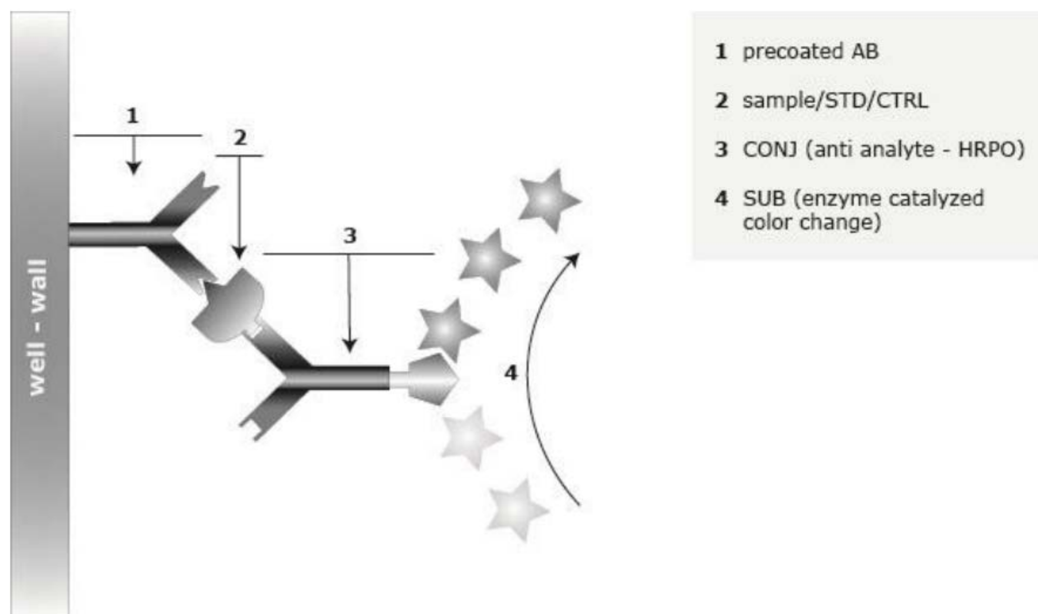
Reconstitute as follows:

Wash buffer: Dilute the concentrate 1:20 (1+19), e.g., 50ml wash buffer + 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 wash buffer to perform the assay.

Standards and Controls: Pipette 300 µL of distilled or deionized 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 standards and controls are stable at -25° C or lower until expiry date stated on the label. Standards and controls are stable for 3 freeze-thaw cycles.

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 precoated 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 color change is detectable with a standard microtiter plate ELISA reader



ASSAY PROTOCOL

All reagents and samples must be at room temperature (18-26° C) before use in the assay.

Mark position for Standard/Sample/Control on the protocol sheet.

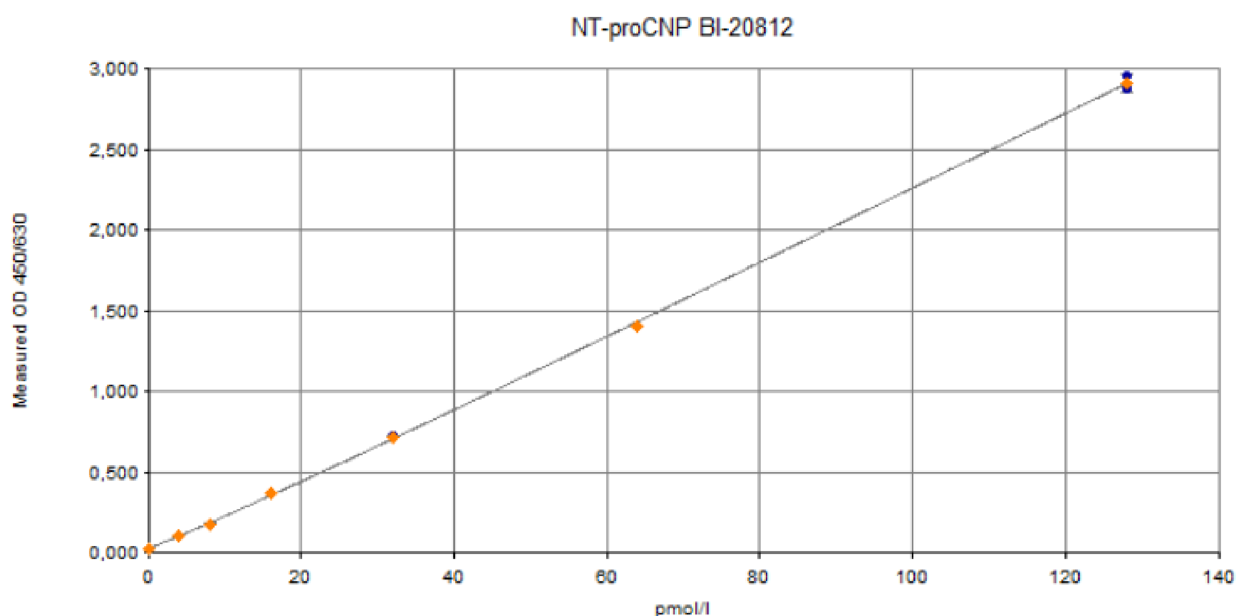
Take microtiter strips out of the aluminum bag, reserve a minimum of one well as Blank. Store unused strips with desiccant at 4° C (2-8° C) in the aluminum bag. Strips are stable until expiry date stated on the label.

1. Pipette 50 µL assay buffer, red cap, into each well.
2. Add 20 µL 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 conjugate, amber cap, into each well and 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 wash buffer, remove remaining wash buffer by hitting plate against paper towel after the final washing step
7. Add 100 µL 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 solution, white cap, into each well, swirl gently
10. Measure absorbance immediately at 450 nm with reference 630 nm, if available

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 standard. 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 must be evaluated by the user. Respective dilution factors must be considered.

TYPICAL STANDARD-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 standard with the highest concentration and the value of the control is in range (see label for target range).

ASSAY CHARACTERISTICS

<table>
<tr><td>Method</td><td colspan="3">Sandwich ELISA, HRP/TMB, 12 x 8 well strips</td></tr>
<tr><td>Sample type</td><td colspan="3">Serum, EDTA plasma, heparin plasma, and citrate plasma. Protocols available for urine, cell culture supernatant, and non-human species</td></tr>
<tr><td>Values for apparently healthy individuals and hospital panel</td><td colspan="3">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>
<tr><td>Standard range</td><td colspan="3">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 colspan="3">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 colspan="3">20 µL/well</td></tr>
<tr><td>Sensitivity</td><td colspan="3">LOD: (0 pmol/L + 3 SD): 0.7 pmol/L; LLOQ: 0.5 pmol/L</td></tr>
<tr><td>Incubation time & temperature</td><td colspan="3">20 min / 3 hr / 30 min, room temperature</td></tr>
<tr><td>Specificity</td><td colspan="3">This assay recognizes endogenous and synthetic human NT-proCNP</td></tr>
<tr><td>Precision</td><td colspan="3">Intra-assay (n=5) ≤ 6%
Inter-assay (n=8) ≤ 7%</td></tr>
<tr><td rowspan="2">Spike/recovery (average recovery
spiked with 64 pmol/L synthetic NT-proCNP)</td><td>Serum (n=6): 101%</td><td colspan="2">Heparin plasma (n=2): 93%</td></tr>
<tr><td>EDTA plasma (n=6): 99%</td><td colspan="2">Citrate plasma (n=2): 100%</td></tr>
<tr><td rowspan="5">Dilution linearity of endogenous NT-proCNP</td><td>Average % expected of dilution</td><td>1+1</td><td>1+3</td></tr>
<tr><td>Serum (n=6)</td><td>99</td><td>98</td></tr>
<tr><td>EDTA plasma (n=6)</td><td>103</td><td>98</td></tr>
<tr><td>Heparin plasma (n=2)</td><td>100</td><td>100</td></tr>
<tr><td>Citrate plasma (n=2)</td><td>96</td><td>92</td></tr>
</table>

PRECISION

Intra-Assay: 2 samples were tested 5 times within 1 assay lot by one operator.

Inter-Assay: 2 samples were tested 8 times in 2 different kit lots by 2 different operators.

Intra-Assay (n=5)	Sample 1	Sample 2	Inter-Assay (n=8)	Sample 1	Sample 2
Mean (pmol/L)	7.9	65.3	Mean (pmol/L)	8.2	64.1
SD (pmol/L)	0.47	1.25	SD (pmol/L)	0.54	1.42
CV	6%	2%	CV (%)	7	2

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.

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 of 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.
- Wear gloves, glasses and lab jacket while performing this assay. Avoid all direct contact with reagents.
- The stop solution contains sulfuric acid, which is irritating to eyes and skin. Irritations are possible – flush with water if contact occurs!

LITERATURE

1. Dynamic response of C-type natriuretic peptide and its aminoterminal propeptide (NTproCNP) to growth hormone treatment in children with short stature. Olney RC et al., Clin Endocrinol, 2016; 85(4):561-568.
2. Serum NT-proCNP levels increased after initiation of GH treatment in patients with achondroplasia/hypochondroplasia. Kubota T et al., Clin Endocrinol (Oxf), 2016; 84(6):845-850.
3. C-type natriuretic peptide in complicated pregnancy: increased secretion precedes adverse events. Reid RA et al., J Clin Endocrinol Metab, 2014; 99(4):1470-1478.
4. Effects of pre-eclampsia and fetal growth restriction on C-type natriuretic peptide. Espiner, E A et al., BJOG, 2015; 122:1236-1243.
5. Prognostic value of circulating amino-terminal pro-C-type natriuretic peptide in critically ill patients. Koch et al., Critical Care, 2011; 15:R45.

6. The prognostic value of concomitant assessment of NT-proCNP, C-reactive protein, procalcitonin and inflammatory cytokines in septic patients. Tomasiuk R et al., Crit Care, 2014; 25;18(3):440.
7. C-Type Natriuretic Peptides in Coronary Disease. Prickett TCR et al., Clin Chem, 2017; 63(1):316-324.
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.

ASSAY PROTOCOL AND CHECKLIST

PREPARATION OF REAGENTS

- ☐ Bring all reagents to room temperature (18-24°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 aluminum bag and mark positions on the protocol sheet.

TEST PROCEDURE

- ☐ **Step 1**: Add 50µl STD/ SAMPLE/ CTRL (standard/ sample/ diluted control) into each well, except blank.
- ☐ **Step 2**: Add 200µl CONJ (Conjugate) into each well, except blank. Swirl gently.
- ☐ **Step 3: Cover tightly and incubate for 4 hours at room temperature (18-24°C) in the dark**.
- ☐ **Step 4**: Aspirate and wash wells with 300µl WASHBUF (wash buffer) five times. Remove remaining buffer by hitting plate against paper towel.
- ☐ **Step 5**: Add 200µl SUB (substrate) into each well.
- ☐ **Step 6: Incubate for 30 minutes at room temperature (18-24°C) in the dark**.
- ☐ **Step 7**: Add 50µl STOP (Stop solution) into each well.
- ☐ **Step 8**: Read Optical Density at 450nm with reference 630nm, if available.

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