

# Human NT-proCNP ELISA Kit

PromoKine

## Instruction Manual

Cat.No. PK-EL-KB20872



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## Intended Use

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This PromoKine Assay is intended for the quantitative determination of NT-proCNP in plasma, serum and cell culture supernatants. It is for *in vitro* research use only.

## Introduction

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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 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).

### *Possible Indications*

- vascular disease
- growth
- skeletal development
- angiogenesis
- sepsis

### **Material Supplied**

<b>Content</b>	<b>Kit Components</b>	<b>Quantity</b>
PLATE	Polyclonal sheep anti NT-proCNP antibody precoated microtiter strips in strip holder packed in aluminium bag with desiccant	12 x 8 tests
WASHBUF	ELISA 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; 4; 8; 16; 32; 64; 128 pmol/l) white caps, lyophilised	7 vials
CTRL	Control A + B, yellow caps, lyophilized, exact concentration see labels	2 vials
CONJ	Conjugate, (sheep anti NT-proCNP-HRPO), amber cap, ready to use	1 x 7 ml
SUB	Substrate (TMB Solution), blue cap, ready to use	1 x 13 ml
STOP	STOP solution, white cap, ready to use	1 x 7 ml

- 1 self-adhesive plastic film
- Instruction manual for use

## Material Required but not Supplied

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- 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

## Reagents and Sample Preparation

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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).

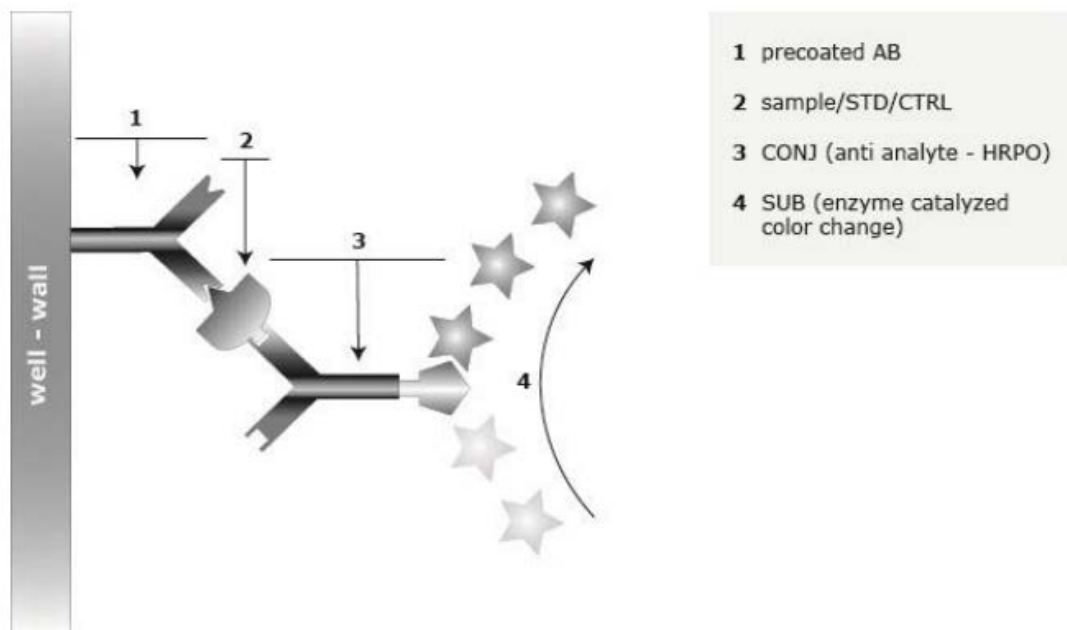
### *Reconstitution /Handling:*

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.

## 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 colour change is detectable with a standard microtiter plate ELISA reader.



## Assay Protocol

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- 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
- Pipette 50 µl ASYBUF (Assay buffer, red cap) into each well.
- Add 20 µl STD/CTRL/SAMPLE (Standard/Control/Sample) in duplicate into respective wells, swirl gently.
- Cover tightly and incubate for 20 minutes at room temperature (18-26°C).
- Add 50 µl CONJ (Conjugate, amber cap) into each well, swirl gently.
- Cover tightly and incubate for 3 hours at room temperature (18-26°C) in the dark.
- 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.*
- *Add 100 µl SUB (Substrate, blue cap) into each well, swirl gently.*
- *Incubate for 30 min at room temperature (18-26°C) in the dark.*
- Add 50 µl STOP (Stop solution, white cap) into each well, swirl gently.
- Measure absorbance immediately at 450 nm with reference 630 nm, if available.

## Calculation of Results

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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.

## Assay Characteristics

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<b>Method:</b>	Sandwich ELISA, HRP/TMB, 12x8-well strips
<b>Sample type:</b>	Serum EDTA plasma, heparin plasma, and citrate plasma. Protocols available for urine, cell culture supernatant and non-human species.
<b>Standard range:</b>	0 – 128 pmol/l
<b>Conversion factor:</b>	1 pg/ml = 0.201 pmol/l 1 pmol/l = 4,985 pg/ml
<b>Sample volume:</b>	20 µl/well
<b>Incubation time, temperature:</b>	20 min / 3 h / 30 min, room temperature
<b>Sensitivity:</b>	LOD: (0 pmol/l + 3 SD): 0.7 pmol/l; LLOQ: 0.5 pmol/l
<b>Specificity:</b>	This assay recognizes endogenous and synthetic human NT-proCNP.



## Precision

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### Inter-Assay (n=8)

Mean (pmol/l)	8.2	64.1
SD (pmol/l)	0.54	1.42
CV (%)	7	2

### Intra-Assay (n=5)

Mean (pmol/l)	7.9	65.3
SD (pmol/l)	0.47	1.25
CV (%)	6	2

## Technical Hints

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- 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

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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  $\leq 0.1\%$  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 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!

## Literature

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- „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.
- „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.
- “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.

- „Effects of pre-eclampsia and fetal growth restriction on C-type natriuretic peptide.” Espiner, E A et al., BJOG, 2015; 122:1236-1243.
- “Prognostic value of circulating amino-terminal pro-C-type natriuretic peptide in critically ill patients.” Koch et al., Critical Care, 2011; 15:R45.
- „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.
- “C-Type Natriuretic Peptides in Coronary Disease.” Prickett TCR et al., Clin Chem, 2017; 63(1):316-324.
- “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.

## Ordering Information

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Product Name	Product Description	Size	Catalog Number
NT-proCNP ELISA Kit, human	Human C-Type Natriuretic Propeptid (NT-proCNP) ELISA Kit	96 Tests	PK-EL-KB20872

***For in vitro research use only.  
Not for diagnostic or therapeutic procedures.***

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