

# Human NT-proANP 1-98 ELISA Kit

PromoKine

## Instruction Manual

Cat.No. PK-EL-KB20892



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## Intended Use

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This PromoKine Assay is intended for the quantitative determination of proANP (1-98) in plasma, serum and cell culture supernatants. It is for *in vitro* research use only.

## Introduction

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Atrial natriuretic peptide is synthesized in atrial myocytes and is stored in secretory granules as a 126 amino acid prohormone. The most important stimulus for the release of the hormone into circulation is stretch of the myocyte fibres. On release the prohormone is split into equimolar amounts of the highly biologically active proANP (99-126), also known as  $\alpha$ -ANP, and the N-terminal part proANP (1-98).  $\alpha$ -ANP is rapidly cleared from the circulation with a half-life of 3-4 minutes. proANP (1-98) has a much longer half-life (60-120 min) which leads to significantly higher concentrations in blood compared to  $\alpha$ -ANP. Thus, circulating levels of proANP (1-98) are less sensitive to the pulsatile secretion of ANP and may better reflect chronic levels of ANP secretion than the rapidly fluctuating levels of  $\alpha$ -ANP. proANP is discussed as valuable marker for e.g. sepsis (Increased plasma levels of NT-proANP and NT-proBNP as markers of cardiac dysfunction in septic patients. Hoffmann U et al. Clin Lab. 2005; 51 (7-8):373-9), or risk stratification in heart failure (Neurohormonal risk stratification for sudden death and death owing to progressive heart failure in chronic heart failure. Berger R et al European Journal of Clinical Investigation, 2005, 35 (1), 24-31).

### *Possible Indications*

- Research studies on heart failure (LVD, CHF etc.)
- Research studies on heart transplanted patients
- Drug therapy monitoring in cardiovascular disease
- Risk assessment in heart failure
- Risk assessment in MI patients with normal NT-proBNP levels
- Monitoring of cardiac resynchronisation therapy

## Material Supplied

Content	Kit Components	Quantity
PLATE	Polyclonal sheep anti proANP microtiterstrips in strip holder packed	12 x 8 tests
WASHBUF	ELISA wash buffer concentrate (20x), natural cap	1 x 50 ml
ASYBUF	Assay Buffer, red cap, ready to use (only for samples above 10 nmol/l !)	1 x 25 ml
CONJ	Conjugate (polyclonal anti-proANP antibody-HRPO), amber cap, ready to use	1 x 22 ml
STD	Standards, synthetic human proANP (1-98) (0;0.63;1.25;2.5;5.0;10.0 nmol/l), white caps, lyophilized	6 vials lyophilized
CTRL	Control, synthetic human proANP (1-98), lyophilized, yellow cap, exact concentration after reconstitution; see label	1 vial lyophilized
SUB	TMB substrate (Tetramethylbenzidine), blue cap, ready-to-use	1 x 22 ml
STOP	Stop solution, sulphuric acid, white cap, ready to use	1 x 7 ml

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

## Material Required but not Supplied

- Precision pipettes calibrated to deliver 10-1000 µl and disposable tips
- ELISA reader for absorbance at 450 nm (or from 450 nm to 620 nm)
- Graph paper or software for calculation of results
- Distilled or deionised water

## Reagents and Sample Preparation

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ProANP in freshly collected blood samples is stable for at least 2.5 hrs at RT (18-24°C). Nevertheless we recommend to perform plasma separation by centrifugation as soon as possible, e.g. 20 minutes at 2,000 x g, preferably at +4°C (2-8°C).

Aliquot and store the acquired plasma samples at -25°C or lower. Samples can be subjected to 3 freeze-thaw cycles.

Lipemic or haemolyzed samples may give erroneous results. Urine or cell culture supernatants are used neat, without any further treatment. Samples should be mixed well before assaying. We recommend duplicates for all values. If samples read higher than STD6, we recommend to dilute with ASYBUF (dilution buffer), e.g.: 1+4 and 1+9, and to re-measure the samples.

The assay can also be used with serum samples under the following conditions:

Serum separation is performed within 1 hour after blood collection. The samples must be tested immediately after separation or must be stored at -25°C or lower, not subjected to more than 2 freeze-thaw cycles. This is due to the lower stability of proANP (1-98) in serum compared to EDTA plasma.

For further information on sample stability please contact our technical and customer service.

### Reconstitution / Handling:

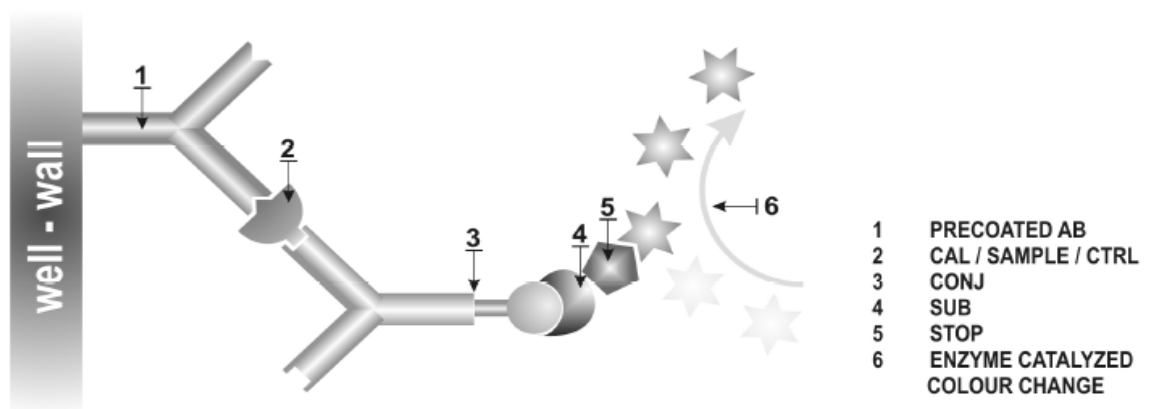
STD (Standard) and CTRL (Control): Pipette 250 µl of distilled or deionised water into the vial. Leave at room temperature (18-24°C) for 10 min. Reconstituted standard and control are stable at -25°C or lower until expiry date on label. Avoid freeze-thaw cycles.

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. The undiluted WASHBUF is stable at 4°C (2-8°C) until expiry date stated on label. The diluted WASHBUF is stable up to one month at 4°C (2-8°C). Only use diluted WASHBUF (Wash buffer) when performing the assay.

## Principle of the Assay

This kit is a sandwich enzyme immunoassay for the determination of proANP in human serum.

In a first step, sample and conjugate (sheep anti human proANP-HRPO) are pipetted into the wells of the microtiter strips, which are pre-coated with polyclonal sheep anti-proANP antibody. proANP present in the sample binds to the pre-coated antibody in the well and forms a sandwich with the conjugate. In the washing step all non-specific unbound material is removed. In a second step, the substrate (TMB Tetramethylbenzidine) is pipetted into the wells. The enzyme catalysed colour change of the substrate is directly proportional to the amount of proANP present in the sample. This colour 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 BLANK/STD/SAMPLE/CTRL (Blank/Standard/Sample/Control) on the protocol sheet.
- Take microtiter strips out of the alu bag, take a minimum of one well as Blank. Store unused strips with desiccant at 2-8°C in the alu bag. Strips are stable until expiry date stated on the label.

- Add 10  $\mu\text{l}$  STD/SAMPLE/CTRL (Standards/Sample/Control) in duplicate into respective well, except blank.
- Add 200  $\mu\text{l}$  CONJ (Conjugate) into each well except blank, swirl gently.
- **Cover tightly and incubate for 3 hours at room temperature (18-24°C) in the dark.**
- Aspirate and wash wells 5x with 300  $\mu\text{l}$  diluted WASHBUF (Wash buffer), remove remaining WASHBUF by hitting plate against paper towel after the latest wash. Add 200  $\mu\text{l}$  SUB (Substrate) into each well.
- **Incubate for 30 minutes at room temperature (18-24°C) in the dark.**
- Add 50  $\mu\text{l}$  STOP (Stop solution) into each well.
- Measure absorbance immediately at 450 nm with reference 620 nm, if available.

## Calculation of Results

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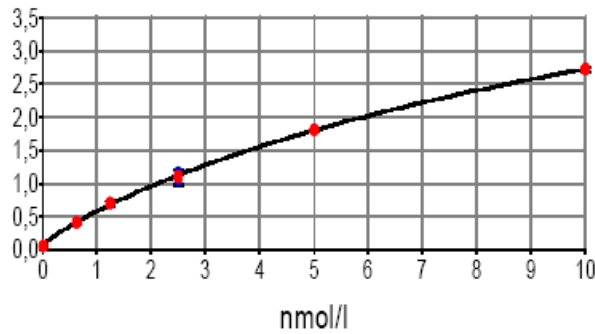
Construct the standard curve from the standard values. Use commercially available software or graph paper. Obtain sample concentration from this standard curve. The assay has been evaluated using a 4PL algorithm. Different curve fitting methods need to be evaluated by the user. Respective dilution factors have to be considered.

The quality control protocol supplied with the kit shows the results of the final release QC for each kit. Data for optical density 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 optical density of 1.00 is obtained for the standard with the highest concentration.

**Example typical STD-curve:**

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## Assay Characteristics

Method:	Sandwich ELISA, HRP/TMB, 12x8-well strips			
Sample type:	Serum, EDTA plasma, heparin plasma, urine, cell culture supernatant			
Standard range:	0 to 10 nmol/l (6 standards and 1 control in a human plasma matrix)			
Conversion factor:	1 nmol/l = 12.7 ng/ml or 1 ng/ml = 0.079 nmol/l (MW: 12.7 kDa)			
Sample volume:	10 µl / well			
Incubation time:	3 h / 30 min			
Sensitivity:	LOD: (0 pmol/l + 3 SD): 0.05 nmol/l			
Specificity:	This assay recognizes endogenous and recombinant human proANP (1-98). The assay also detects mouse and rat proANP (1-98).			
Cross-reactivity:	proANP (1-30) <1%, proANP (31-67) <1%, proANP (79-98) <1%, alpha ANP (99-126) <1%, proBNP (8-29) <1%, proBNP (32-57) <1%, proCNP (1-19) <1%, proCNP (30-50) <1%, proCNP (51-97) <1%			
Precision:	Intra-assay (n=5) ≤ 5% , Inter-assay (n=3) ≤ 9%			
Spike/Recovery (average recovery spiked with 4 nmol/l rec. proANP):	EDTA plasma (n=4): 89%			
Dilution linearity (average recovery of expected proANP after a 1+1; 1+3; 1+7 dilution):	Dilution:	1+1	1+3	1+7
	EDTA plasma (n=3)	90%	87%	79%
Values from apparently healthy individuals:	Median EDTA plasma (n=53): 1.45 nmol/l Each laboratory should establish its own reference range for the samples under investigation. Do not change sample type during the study.			



## Precision

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Intra-Assay (n=5)	Sample 1	Sample 2
Mean (nmol/l)	1.07	7.58
SD (nmol)	0.05	0.16
CV (%)	5	2

Inter-Assay (n=3)	Sample 1	Sample 2
Mean (nmol/l)	1.11	7.24
SD (nmol)	0.1	0.37
CV (%)	9	5

## 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 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.

## Precautions

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- 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.
- All liquid reagents contain  $\leq 0.01\%$  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.
- Sulfuric acid is irritating to eyes and skin. Flush with water if contact occurs. Avoid contact with skin and mucous. Irritations are possible – Flush with water after contact!

## Literature

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- Neurohormonal risk stratification for sudden death and death owing to progressive heart failure in chronic heart failure. Berger R. et al. *European Journal of Clinical Investigation*, 2005, 35 (1), 24-3.
- Increased plasma levels of NT-proANP and NT-proBNP as markers of cardiac dysfunction in septic patients. Hoffmann U. et al. *Clin. Lab.* 2005, 51(7-8), 373-9.
- Risk assessment in patients with unstable angina/non-ST-elevation myocardial infarction and normal N-terminal pro-brain natriuretic peptide levels by N-terminal pro-atrial natriuretic peptide. Jarai R. et al. *European Heart Journal* 2004, 26 (3), 250-256.
- Atrial and brain natriuretic peptides as markers of response to resynchronisation therapy. Molhoek S. G. et al. *Heart* 2004, 90, 97-98.

- N-terminal proatrial natriuretic peptide in primary care: relation to echocardiographic indices of cardiac function in mild to moderate cardiac disease. Hall C. et al. Int. J. Cardiol. 2003 Jun, 89(2-3), 197-205.
- Prognostic value of two-dimensional echocardiography and N-terminal proatrial natriuretic peptide following an acute myocardial infarction. Assessment of baseline values (2-7 days) and changes at 3 months in patients with a preserved systolic function. Otterstad JE et al., Eur Heart J 2002 Jul; 23(13):1011-20.

### Assay Protocol and Checklist

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- 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 alu bag and mark positions on the protocol sheet.
- Add 10 µl STD/ SAMPLE/CTRL (standard/sample/control) into each well, except blank.
- Add 200 µl CONJ (Conjugate), except blank. Swirl gently.
- **Cover tightly and incubate for 3 hrs at room temperature (18-24°C) in the dark.**
- Aspirate and wash wells with 300 µl WASHBUF (Wash buffer) five times. Remove remaining buffer by hitting plate against paper towel.
- Add 200 µl SUB (Substrate) into each well.
- **Incubate for 30 minutes at room temperature (18-24°C) in the dark.**
- Add 50 µl STOP (Stop solution) into each well.
- Read Optical Density at 450 nm with reference 620 nm, if available.

## Ordering Information

Product Name	Product Description	Size	Catalog Number
NT-proANP 1-98 ELISA Kit, human	Human Atrial Natriuretic proPeptid (proANP 1-98) ELISA Kit	96 Tests	PK-EL-KB20892

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

**PromoCell GmbH**  
Sickingenstr. 63/65  
69126 Heidelberg  
Germany

USA / Canada

Phone: 1 – 866 – 251 – 2860 (toll free)

Fax: 1 – 866 – 827 – 9219 (toll free)

Deutschland

Telefon: 0800 – 776 66 23 (gebührenfrei)

Fax: 0800 – 100 83 06 (gebührenfrei)

France

Téléphone: 0800 90 93 32 (ligne verte)

Téléfax: 0800 90 27 36 (ligne verte)

United Kingdom

Phone: 0800 – 96 03 33 (toll free)

Fax: 0800 – 169 85 54 (toll free)

Other Countries

Phone: +49 6221 – 649 34 0

Fax: +49 6221 – 649 34 40

Email: [info@promokine.info](mailto:info@promokine.info)

[www.promocell.com](http://www.promocell.com)