Product Manual

proANP (1-98)

Catalog #: ENZ-KIT153

ENZYME IMMUNOASSAY FOR THE QUANTITATIVE DETERMINATION OF HUMAN proANP(1-98) IN EDTA PLASMA, HEPARIN PLASMA, SERUM, URINE OR CELL CULTURE SUPERNATANTS.

1x 96 wells

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Product Manual

CONTENTS

Please read entire booklet before proceeding with the assay.

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

Please contact Enzo Life Sciences Technical Support if necessary.

INTRODUCTION

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 fibers. On release the prohormone is split into equimolar amounts of the highly biologically active proANP (99-126), also known as a- ANP, and the N-terminal part proANP (1-98). a-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 a-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 a-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 patients
- Risk assessment in MI patients with normal NT-proBNP levels
- Monitoring of cardiac resynchronization therapy

MATERIALS SUPPLIED

CONT	KIT COMPONENTS	QUANTITY
PLATE	Polyclonal sheep anti proANP pre-coated microtiter strips in stripholder	12 x 8 tests
WASHBUF	Wash buffer concentrate 20x, natural cap	1 x 50ml
ASYBUF	Assay Buffer, red cap, ready-to-use (only for samples above 10nmol/l!)	1 x 25ml
STD	Standards, synthetic human proANP (1-98) (0;0.63;1.25;2.5;5;10nmol/l), white caps, lyophilized	6 vials
CTRL	Control, synthetic human proANP (1-98), lyophilized, yellow cap, see label for exact concentration after reconstitution	1 vial
CONJ	Conjugate (sheep polyclonal anti proANP antibody-HRPO), amber cap, ready-to-use	1 x 22ml
SUB	Substrate, (TMB solution), blue cap, ready-to-use	1 x 22ml
STOP	Stop solution, sulphuric acid, white cap, ready-to-use	1 x 7ml

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 10-1000µl and disposable tips
- Distilled or deionized water
- Refrigerator with 4°C (2-8°C)
- ELISA reader for absorbance at 450nm (reference 630nm)
- Graph paper or software for calculation of results

REAGENTS AND SAMPLE PREPARATION

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 min 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 hemolyzed 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 hr 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.

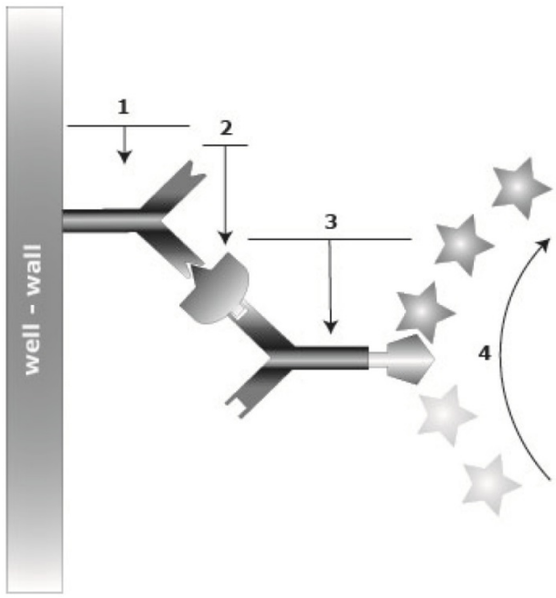
Reconstitution/Handling:

STD (Standard) and CTRL (Control): Pipette 250µl of distilled or deionized 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. 50ml WASHBUF + 950ml 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 catalyzed color change of the substrate is directly proportional to the amount of proANP present in the sample. This color change is detectable with a standard microtiter plate ELISA reader.



1 precoated AB
2 sample/STD/CTRL
3 CONJ (anti analyte - HRPO)
4 SUB (enzyme catalyzed color change)

ASSAY PROTOCOL

All reagents and samples must be at room temperature (18-24°C) before use in the assay.

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

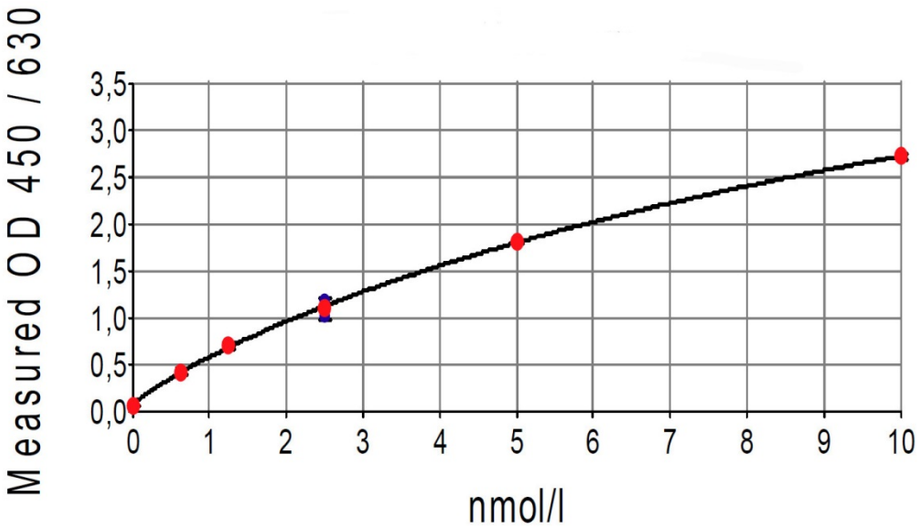
Take microtiter strips out of the aluminum bag, reserve a minimum of one well as Blank. Store unused strips with desiccant at 4°C (2-8°C) in the aluminum bag. Strips are stable until expiry date stated on the label.

1. Add 10µl STD/SAMPLE/CTRL (Standards/Sample/Control) in duplicate into respective well.
2. Add 200µl CONJ (Conjugate, amber cap) into each well, except blank, swirl gently.
3. **Cover tightly and incubate 4 hours at room temperature (18-24°C) in the dark**.
4. Aspirate and wash wells 5x with 300µl diluted WASHBUF (Wash buffer). Remove remaining WASHBUF by hitting plate against paper towel after the last wash.
5. Add 200µl SUB (Substrate, blue cap) into each well, swirl gently.
6. **Incubate for 30 minutes at room temperature (18-24°C) in the dark**.
7. Add 50µl STOP (Stop solution, white cap) into each well, swirl gently.
8. Measure absorbance immediately at 450nm with reference 630nm, if available.

CALCULATION OF RESULTS

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.

TYPICAL STANDARD-CURVE



The quality control 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 1.00 or higher is obtained for the standard with the highest concentration.

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 10nmol/l (6 standards and 1 control in a human plasma matrix)			
Conversion factor:	1nmol/l = 12.7ng/ml or 1ng/ml = 0.079nmol/l (MW: 12.7 kDa)			
Sample volume:	10µl / well			
Incubation time:	4h / 1h / 30min			
Sensitivity:	LOD: (0 pmol/l + 3 SD): 0.05nmol/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 4nmol/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): 1.45nmol/l Each laboratory should establish its own reference range for the samples under investigation. Do not change sample type during the study.			

No hook-effect was observed up to a concentration of 80nmol/l.

PRECISION

Intra-Assay: 2 samples of known concentrations were tested 5 times in 1 kit lot by 1 operator.

Inter-Assay: 2 samples of known concentrations were tested 3 times within 3 different kit assay lots by 2 different operators.

Intra-Assay (n=5)	Sample 1	Sample 2	Inter-Assay (n=3)	Sample 1	Sample 2
Mean (nmol/l)	1.07	7.58	Mean (nmol/l)	1.11	7.24
SD (nmol/l)	0.05	0.16	SD (nmol/l)	0.10	0.37
CV%	5%	2%	CV%	9%	5%

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 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 ≤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.
- Sulfuric acid is irritating to eyes and skin. Avoid contact with skin and mucous. Irritations are possible – flush with water if contact occurs!

Handle with care

LITERATURE

1. 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), 24-33
2. 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), 373-379
3. Risk assessment in patients with unstable angina/non-ST-elevation myocardial infarction and normal N-terminal proBrain Natriuretic Peptide levels by N-terminal pro-atrial natriuretic peptide. Jarai R. et al. European Heart Journal 2004, 26 (3), 250-256
4. Atrial and brain natriuretic peptides as markers of response to resynchronization therapy. Molhoek S. G. et al. Heart 2004, 90, 97-98
5. 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
6. 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):1011-1020

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 10µl STD/SAMPLE/CTRL (standard/ sample/ control) into each well.
- **Step 2**: Add 200µl CONJ (conjugate). Swirl gently.
- **Step 3: Cover tightly and incubate for 3 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. Swirl gently.
- **Step 8**: Read Optical Density at 450nm with reference 630nm, if available.

NOTES

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