NT-proBNP

(EN) ENZYME IMMUNOASSAY FOR THE QUANTITATIVE DETERMINATION OF NT-proBNP IN HUMAN SERUM
CAT. NO. SK-1204 12 X 8 TESTS

(DE) ENZYME IMMUNOASSAY FÜR DIE QUANTITATIVE BESTIMMUNG VON NT-proBNP IN HUMANEN SERUM PROBEN
KAT. NR. SK-1204 12 X 8 TESTE

(FR) KIT DE DOSAGE IMMUNOENZYMATIQUE POUR LA DETERMINATION DE LA CONCENTRATION EN NT-proBNP DANS LE SERUM HUMAIN
REF. SK-1204 12 X 8 TESTS

(IT) SAGGIO IMMUNOENZIMATICO PER LA DETERMINAZIONE QUANTITATIVA DI NT-proBNP NEL SIERO UMANI
CODICE. SK-1204 12 X 8 DERMINAZIONI

CE

BIOMEDICA

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1) INTRODUCTION

BNP is mainly expressed by ventricular myocardium in response to volume overload and increased filling pressure. BNP has a cleavable signal sequence. Mature BNP consists of 108 amino acids (proBNP or BNP-108), and undergoes cleavage resulting in physiologically active BNP-32 and additional C-terminal fragments (cf. http://www.uniprot.org/uniprot/P16860#PRO_0000001532), along with a physiologically inactive N-terminal peptide comprising amino acids 1-76, which is further degraded proteolytically.

BNP has a key role in cardiovascular homeostasis with biological actions including natriuresis, diuresis, vasorelaxation, and inhibition of renin and aldosterone secretion. A high concentration of BNP in the bloodstream is indicative of heart failure.

Areas of Interest

- Cardiac impairment, acute myocardial infarction, (left ventricular dysfunction)
- Renal failure
- Obesity and diabetes
- Various forms of secondary hypertensions
- Therapy monitoring of heart failure patients

2) CONTENTS OF THE KIT

CONT	KIT COMPONENTS	QUANTITY
PLATE	polyclonal sheep anti NT-proBNP antibody coated microtiter strips in stripholder packed in alu bag with desiccant	12 x 8 tests
WASHBUF	Wash buffer, 20x concentrated , clear cap	1 x 50 ml
STD	Standards, synthetic human NT-proBNP (0/10/40/160/640 pmol/l), lyophilised, white caps	5 vials lyophilised
CTRL	Control, synthetic human NT-proBNP, lyophilised, yellow cap, exact concentration after reconstitution see label	1 vial lyophilised
CONJ	Conjugate, (sheep anti human NT-proBNP-HRPO), red dye, brown cap, ready to use	1 x 22 ml
SUB	Substrate (TMB solution), blue cap, ready to use	1 x 22 ml
STOP	Stop solution, sulphuric acid, white cap, ready to use	1 x 7 ml

3) ADDITIONAL MATERIAL ADDED TO THE KIT

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

4) MATERIAL AND EQUIPMENT REQUIRED BUT NOT SUPPLIED

- Precision pipettes calibrated to deliver 50-500 µl and disposable tips
- ELISA reader for absorbance at 450 nm (or from 450 nm to 630 nm)
- Graph paper or software for calculation of results
- Plate washer is recommended for washing
- Distilled or deionised water

5) REAGENTS AND SAMPLE PREPARATION

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

Sample preparation:

NT-proBNP is stable in whole blood for several hours at room temperature (18-26°C). Nevertheless we recommend separating serum by centrifugation as soon as possible, e.g. 20 min at 2,000 x g, preferably at 4°C (2-8°C). Serum can be stored at 4°C (2-8°C) up to two days. For long term storage, aliquot the acquired 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. Samples with values above STD5 (640 pmol/l) can be diluted with STD1 or NT-proBNP negative human serum.

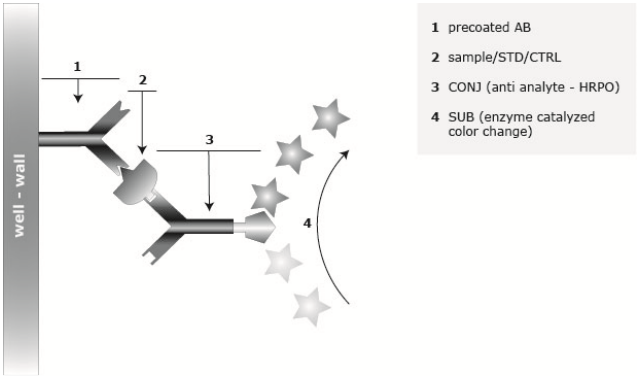
For further information on sample stability and assay performance characteristics please visit our website www.bmgrp.com (s. Validation Data) or contact our customer service by e-mail export@bmgrp.com or by phone +43/ 1/ 29107-45.

Reconstitution/Handling:

- STD (Standard): Pipette 500 µl of distilled or deionised water into each vial. Leave at room temperature (18-26°C) for 10 min. Swirl gently. The standard concentration is printed on the label. Reconstituted standard is stable at -25°C until expiry date. Avoid freeze-thaw cycles.
- CTRL (Control): Pipette 500 µl of distilled or deionised water to the vial. Leave at room temperature (18-26°C) for 10 min. Swirl gently. The final concentration is stated on the label. Reconstituted control is stable at -25°C until expiry date stated on label. Avoid freeze-thaw cycles.
- WASHBUF (Wash buffer): Dilute the concentrate 1:20 (e.g. 50 ml WASHBUF + 950 ml distilled water). Crystals in the buffer concentrate will dissolve at room temperature (18-26°C). The undiluted WASHBUF is stable at 4°C (2-8°C) until expiry date stated on label. The diluted WASHBUF is stable at 4°C (2-8°C) up to one month. Use only diluted WASHBUF (Wash buffer) for the assay.

6) PRINCIPLE OF THE ASSAY

This kit is a sandwich enzyme immunoassay for the determination of NT-proBNP in human serum.
In a first step, sample and conjugate (sheep anti human NT-proBNP-HRPO) are pipetted into the wells of the microtiter strips, which are pre-coated with polyclonal sheep anti NT-proBNP antibody. NT-proBNP present in the sample binds to the pre-coated antibody in the well and forms a sandwich with the detection antibody. 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 NT-proBNP present in the sample. This colour change is detectable with a standard microtiter plate ELISA reader.



7) ASSAY PROTOCOL

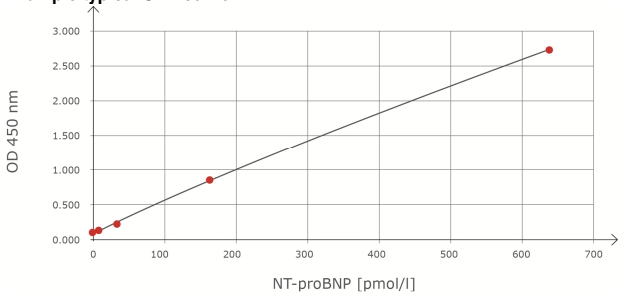
All reagents and samples must be at room temperature (18-26°C) before use in the assay.
Mark position for STD/SAMPLE/CTRL (Standard/Sample/Control) on the protocol sheet.
Take microtiter strips out of the alu bag. Store unused strips with desiccant at 4°C (2-8°C) in the alu bag. Strips are stable until expiry date stated on the label.

1. Add 50 µl STD/SAMPLE/CTRL (Standards/Sample/Control) in duplicate into respective well.
2. Add 200 µl CONJ (Conjugate) into each well, swirl gently.
3. Cover tightly and incubate 3 hours at room temperature (18-26°C).
4. Aspirate and wash wells 5x with 300 µl diluted WASHBUF (Wash buffer), remove remaining WASHBUF by hitting plate against paper towel after the last wash.
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). 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.
Samples with values above STD5 (640 pmol/l) can be diluted with STD1 or NT-proBNP negative human serum and re-assayed. Dilution factors must be taken into consideration for calculation of the sample concentrations.

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 STD5 and the value of the CTRL is in range (target range see label).

9) ASSAY CHARACTERISTICS

Values of apparently healthy individuals:	Median (serum, n = 93): 3.0 pmol/l Each laboratory should establish its own reference data.
Conversion factor pmol/l to pg/ml	1 pmol/l = 8.475 pg/ml refers to NT-proBNP (1-76) that is detected by the ELISA
Standard range:	0 to 640 pmol/l
Sample volume:	50 µl human serum
Detection Limit:	(0 pmol/l + 3 SD) 3 pmol/l
Incubation time:	3 hours / 30 min

10) PRECISION

Experiment:
Intra-assay: 2 samples of known concentrations were tested 3 times in 1 assay by 1 operator.
Inter-assay: 2 samples of known concentrations were tested 8 times in 2 assays by different operators.

Intra-assay (n=3)	**Sample 1**	**Sample 2**	**Inter-assay (n=8)**	**Sample 1**	**Sample 2**
Mean (pmol/l)	60.2	35.2	Mean (pmol/l)	52.1	108.1
SD (pmol/l)	2.0	0.9	SD (pmol/l)	1.7	7.9
CV (%)	4	3	CV (%)	3	7

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 various 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.

12) PRECAUTIONS

All test components of human source were tested 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, which 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.

13) LITERATURE

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