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
CTRL	Controls, synthetic human NT-proBNP, lyophilised, yellow cap, exact concentrations after reconstitution see labels	2 vials
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 (630 nm reference optional)
- Graph paper or software for calculation of results
- Plate washer is recommended for washing, alternatively: multichannel pipette or dispenser
- Refrigerator with 4°C (2-8°C)
- 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 EDTA plasma or serum by centrifugation as soon as possible, e.g. 20 min at 2,000 x g, preferably at 4°C (2-8°C). EDTA plasma or serum can be stored at 4°C (2-8°C) up to two days. For long term storage, aliquot the acquired samples and store them at -25°C or lower. Samples can be subjected to 3 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 STD5 (640 pmol/l) can be diluted with STD1 or NT-proBNP negative human serum.

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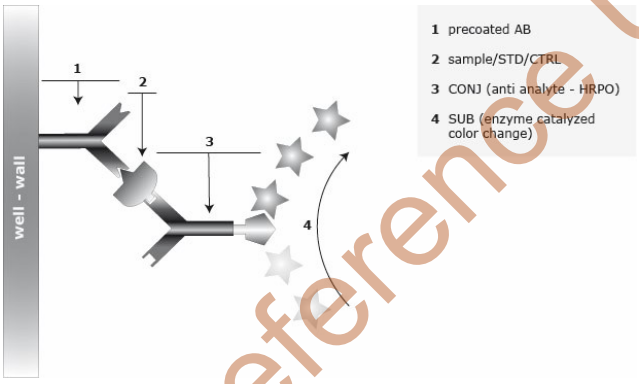
For further information on sample stability and assay performance characteristics please visit our website www.bmgrp.com (s. Validation Data) or contact our customer service by e-mail info@bmgrp.com or by phone +43/ 1/ 29107-45.

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 each 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 or EDTA plasma. 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 conjugate (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 strongly tapping plate against paper towel after the last wash.
5. Add 200 µl SUB (Substrate) into each well, swirl gently.
6. Incubate for 30 min at room temperature (18-26°C) in the dark.
7. Add 50 µl STOP (Stop solution) into each well, swirl gently.
8. Measure absorbance immediately at 450 nm with reference 630 nm, if available.

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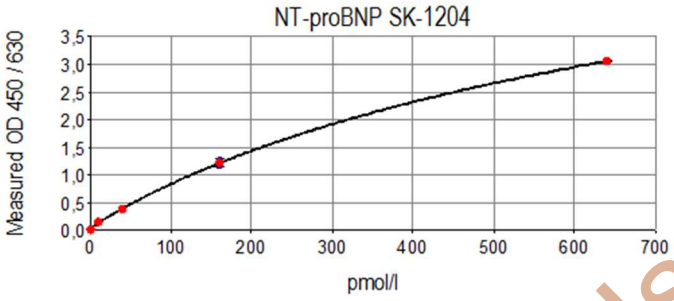
8) CALCULATION OF RESULTS

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 considered when calculating the final 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 values of the CTRLs are in range (target ranges see labels).

9) ASSAY CHARACTERISTICS

Method:	Sandwich ELISA, HRP/TMB, 12x8-well strips		
Antibodies/Standard:	Capture antibody: polyclonal sheep anti human NT-proBNP antibody, specifically binding to amino acids 31-57 of proBNP. Detection antibody: polyclonal sheep anti human NT-proBNP antibody, conjugated to peroxidase, specifically binding to amino acids 8-29 of proBNP. Standard material: synthetic human NT-proBNP (1-76).		
Sample type:	Human serum, EDTA plasma		
Standard range:	0 to 640 pmol/l (0/10/40/160/640 pmol/l)		
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		
Sample volume:	50 µl / well		
Incubation time:	3 hours / 30 min		
Sensitivity:	LOD (0 pmol/l + 3SD): 3.0 pmol/l; LLOQ: 3.3 pmol/l		
Specificity:	This assay recognizes endogenous (natural) and recombinant human NT-proBNP (1-76).		
Precision:	Intra-assay (n=3) ≤ 4%, Inter-assay (n=8) ≤ 7%		
Spike/Recovery (average R spiked with rec. NT-proBNP)		Spike 80 pmol/l	Spike 320 pmol/l
	Serum (n=4)	99%	108%
	EDTA Plasma (n=4)	94%	93%
Dilution linearity (average R of expected NT-proBNP after a 1+1 dilution)	Serum (rec. NT-proBNP): 117%		Serum (endog. NT-proBNP): 82%
	EDTA plasma (rec. NT-proBNP): 84%		EDTA plasma (endog. NT-proBNP): 84%
Values of apparently healthy individuals:	Median (serum, n=70): 5.8 pmol/l Median (EDTA plasma, n=28): 5.6 pmol/l Each laboratory should establish its own reference data.		

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

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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, HCV-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 950 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

1. Utility of the Amino-Terminal Fragment of Pro Brain Natriuretic Peptide in Plasma. For the Evaluation of Cardiac Dysfunction in elderly Patients in Primary Health Care. Alehagen U et al., Clin Chem, 49:8; 1337-1346 (2003).
2. Head-to-head comparison of N-terminal pro-brain natriuretic peptide, brain natriuretic peptide and N-terminal pro-atrial natriuretic peptide in diagnosing left ventricular dysfunction. Hammerer-Lercher et al., Clin Chim Acta, 310(2),193-197 (2001).
3. Prognostic evaluation of neurohumoral plasma levels before and during beta-blocker therapy in advanced left ventricular dysfunction. Stanek B. et al., JACC, 38, 436-442 (2001).
4. Pulmonary arterial hypertension in rheumatic mitral stenosis: does it affect right ventricular function and outcome after mitral valve replacement? Pande S et al., Interact CardioVasc Thorac Surg, 9: 421-425 (2009).
5. Prognostic impact of body mass index in patients undergoing coronary artery bypass surgery. Sung SH et al., Heart, 97: 648-654 (2011).
6. Effect of piboserod, a 5-HT4 serotonin receptor antagonist, on left ventricular function in patients with symptomatic heart failure. Kjekshus JK et al., Eur J Heart Fail, 11: 771-778 (2009).
7. Plasma pro-B-type natriuretic peptide in the general population: screening for left ventricular hypertrophy and systolic dysfunction. Goetze JP et al., Eur Heart J, 27: 3004-3010 (2006).

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SYMBOLS

Expiry date / Verfallsdatum / Date de péremption / Data di scadenza /Fecha de caducidad / Data de validade / Uiterste gebruiksdatum / Udløbsdato / Utgångsdatum / Termin Ważności / Lejárati idő / Doba exspirácie / Doba exspirace

Consider instructions for use / Bitte Gebrauchsanweisung beachten / Consultez la notice d'utilisation / Consultare le istruzioni per l'uso / Consulte las instrucciones de utilización / Consulte as instruções de utilização / Raadpleeg de gebruiksaanwijzing / Se brugsanvisningen / Läs anvisningarna före användning / Proszę przeczytać instrukcję wykonania / Vegyük figyelembe a használati utasításban foglaltakat / Postupujte podľa pokynov na použitie / Postupujte dle návodu k použití

In vitro Diagnostic Medical Device (for in Vitro Diagnostic Use) / In vitro Diagnostikum (zur In-vitro-Diagnostik) / Dispositif médical de diagnostic in vitro (Pour usage diagnostique in vitro) / Dispositivo medico per diagnostica in vitro (per uso diagnostico in vitro) / Dispositivo médico de diagnóstico in vitro (para uso diagnóstico in vitro) / Dispositivo médico para diagnóstico in vitro (Para utilização de diagnóstico "in vitro") / Medisch hulpmiddel voor diagnostiek in vitro (Voor diagnostisch gebruik in vitro) / Medicinsk udstyr til in vitro-diagnostik (Udelukkende til in vitro diagnostisk anvendelse) / Medicinteknisk produkt avsedd för in vitro-diagnostik (För in vitro-diagnostiskt bruk) / Wyrób medyczny do Diagnostyki In Vitro / In vitro orvosdiagnosztikai termék / In vitro diagnostický zdravotnícky materiál (určené pre diagnostiku „in vitro“) / In vitro diagnostický zdravotnicky materiál (určeno pro diagnostiku „in vitro“)

Lot-Batch Number / Charge-Chargennummer / Lot-Code du lot / Lotto-Numero di lotto / Lote-Código de lote / Lote-Código do lote / Lot-Partijnummer / Lot-Batchkode / Lot-Satskod / Numer serii / Lot-Batch szám / Číslo šarže / Číslo šarže

Manufactured by / Hergestellt von / Fabriqué par / Prodotto da / Fabricado por / Fabricado por / Vervaardigd door / Fabrikation af / Tillverkad av / Wyprodukowane pr / Gyártotta / Vyrobené / Vyrobeno

Catalogue Number / Bestellnummer / Numéro de référence / Numero di riferimento / Número de referencia / Número de referência / Referentienummer / Referencenummer / Katalognummer / Numer katalogowy / Katalógusszám / Katalógové číslo / Katalogové číslo

Store at between / Lagerung bei zwischen / Conserver à entre / Conservare a tra / Conservar a temp. entre / Armazene a entre / Bewaar bij tussen / Opbevares mellem / Förvaras vid / Przechowywać w / Tároljuk …… között / Skladujte v rozsahu / Skladujte v rozmezí

Contains sufficient for x tests / Inhalt ausreichend für x Tests / Contient suffisant pour x tests / Contenuto sufficiente per x test / Contiene suficiente para x pruebas / Contém suficiente para x testes / Bevat voldoende voor x bepalingen / Indeholder tilstrækkeligt til x prøver / Innehållet räcker till x analyser / Zawartość na x testów / Tartalma X teszt elvégzésére elegendő / Obsahuje materiál pre x testov / Obsahuje materiál pro x testů

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SK-1204 NT-proBNP
ASSAY PROTOCOL AND CHECKLIST

PREPARATION OF REAGENTS:

- ❑ Bring all reagents to room temperature (18-26°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.

TEST PROCEDURE:

- ❑ Step1) Add 50 µl STD/ SAMPLE/CTRL (standard/sample/control) in duplicate into respective well.
- ❑ Step 2) Add 200 µl CONJ (Conjugate) into each well. Swirl gently.
- ❑ **Step 3) Cover tightly and incubate for 3 hours at room temperature (18-26°C).**
- ❑ Step 4) Aspirate and wash wells 5x with 300 µl diluted WASHBUF (Wash buffer). Remove remaining buffer by strongly tapping plate against paper towel after last wash.
- ❑ Step 5) Add 200 µl SUB (Substrate) into each well. Swirl gently.
- ❑ **Step 6) Incubate for 30 minutes at room temperature (18-26°C) in the dark.**
- ❑ Step 7) Add 50 µl STOP (Stop solution) into each well. Swirl gently.
- ❑ Step 8) Read Optical Density at 450 nm with reference 630 nm, if available.

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