

# ***BNP FRAGMENT EIA***

(EN) ENZYME IMMUNOASSAY FOR THE QUANTITATIVE DETERMINATION OF BNP FRAGMENT  
IN HUMAN SERUM, CITRATE PLASMA, EDTA PLASMA OR HEPARIN PLASMA  
CAT. NO. BI-20852W 12 X 8 TESTS

FOR RESEARCH USE ONLY  
NOT FOR USE IN DIAGNOSTIC PROCEDURES

rev.no. 190111 (replacing 180605)

This kit was developed and manufactured by:

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## **CONTENT / INHALT**

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*Detailed information on the assay characteristics including the validation data can be found on our website.*

**[www.bmgrp.com](http://www.bmgrp.com)**

## 1) INTRODUCTION

The natriuretic peptides are members of a family of structurally similar but genetically distinct peptide hormones, consisting of atrial-, brain-, and C-type (ANP, BNP, and CNP, respectively). ANP and BNP preferentially bind to a membrane-bound guanylyl cyclase (GC) receptor called GC-A or NPR1, whereas CNP is the physiological ligand for GC-B (NPR2). The natriuretic peptides play an important role in the regulation of cardiovascular and renal homeostasis and in the regulation of fatty acid metabolism and body weight.

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](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 fragments in the circulation are therefore very heterogeneous.

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.

The discovery of natriuretic peptides identified an endocrine system that contributes to diuresis and vascular tone. The biology, biochemistry and the pathophysiological role of natriuretic peptides are described in several reviews.

### Areas of Interest

- Cardiac impairment, acute myocardial infarction, (left ventricular dysfunction)
- Renal failure
- Obesity and diabetes
- Various forms of secondary hypertension

## 2) CONTENTS OF THE KIT

CONT	KIT COMPONENTS	QUANTITY
PLATE	polyclonal anti BNP fragment (8-29) antibody coated microtiter strips in strip holder packed in aluminium bag with desiccant	12 x 8 tests
WASHBUF	Wash buffer, 20x concentrated, natural cap	1 x 50 ml
ASYBUF	Assay buffer, red cap, ready to use	1 x 20 ml
STD	Standard 1-7, synthetic human BNP fragment (0; 200; 400; 800; 1,600; 3,200; 6,400 pmol/l), white cap, lyophilised	7 vials lyophilised
CTRL	Control, yellow cap, synthetic human BNP fragment, lyophilised exact concentration after reconstitution see label	1 vial lyophilised
CONJ	Conjugate, (synthetic BNP fragment -HRPO), red dye, amber cap, ready to use	1 x 6 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 IN THE KIT

- 2 self-adhesive aluminium films
- Quality control protocol
- Protocol sheet
- Instruction for use

## 4) MATERIAL AND EQUIPMENT REQUIRED BUT NOT SUPPLIED

- Precision pipettes calibrated to deliver 30 µl, 50 µl, 150 µl, 200 µl, 300 µl and disposable tips
- Distilled or deionised water
- Plate washer is recommended for washing, alternative multichannel pipette or manifold dispenser
- Refrigerator with 4°C (2-8°C)
- ELISA reader capable of measuring absorbance at 450 nm (with correction wavelength at 630 nm)
- Graph paper or software for calculation of result

## 5) REAGENTS AND SAMPLE PREPARATION

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

### Sample preparation:

Collect venous blood samples by using standardized blood collection tubes for serum or plasma. BNP fragments are stable in whole blood, serum or plasma for several hours at room temperature or 4°C (2-8°C). Nevertheless perform serum and plasma separation by centrifugation according to supplier's instructions of the blood collection devices as soon as possible.

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 highest STD could be diluted with STD1 (0 pmol/l) or BNP fragment negative human serum.

For further information on sample stability please visit our website [www.bmgrp.com](http://www.bmgrp.com) validation data or contact our customer service by e-mail [info@bmgrp.com](mailto:info@bmgrp.com) or by phone +43/ 1/ 29107-45.

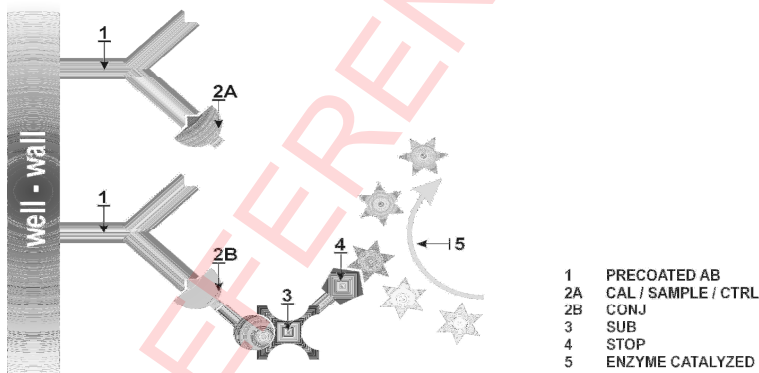
### Reconstitution/Handling:

**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. 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 when performing the assay.

**STD (Standard):** Pipette 200 µl of distilled or deionised water into each vial. Leave at room temperature (18-26°C) for 20 min. Swirl gently. The standard concentration is printed on the label. Reconstituted standard is stable at -25°C or lower until expiry date. Avoid freeze-thaw cycles.

**CTRL (Control):** Pipette 200 µl of distilled or deionised water to the vial. Leave at room temperature (18-26°C) for 20 min. Swirl gently. The final concentration is stated on the label. Reconstituted control is stable at -25°C or lower until expiry date stated on label. Avoid freeze-thaw cycles.

## 6) PRINCIPLE OF THE ASSAY



## 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 aluminium bag. Unused strips can be stored with desiccant in the aluminium bag at +4°C (2-8°C) until the expiry date.

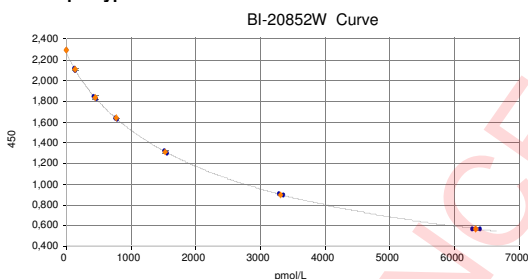
1. Add 150 µl ASYBUF (Assay buffer) into all wells.
2. Add 30 µl STD/ SAMPLE/ CTRL (Standards/Sample/Control) in duplicate into respective well, swirl gently.
3. Add 50 µl CONJ (Conjugate) into each well, swirl gently.

4. Cover tightly and incubate overnight (16-25 hours) at 4°C (2-8°C) in the dark.
5. 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.
6. Add 200 µl SUB (Substrate) into each well.
7. Incubate for 20 min at room temperature (18-26°C) in the dark.
8. Add 50 µl STOP (Stop solution) into each well, swirl gently.
9. 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. Respective dilution factors have to be considered.

### Example typical STD-curve:



The quality control (QC) protocol supplied with the kit shows the results of the final release QC for each lot 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 1.5 or more is obtained for STD 0 and the value of the CTRL is in range (target range see label).

## 9) ASSAY CHARACTERISTICS

Values from apparently healthy individuals (n=76):	In a panel of blood donors the median was 392 pmol/l. Each laboratory has to establish its own reference range for the samples under investigation.
Standard range:	0 to 6,400 pmol/l
Sample volume:	30 µl human serum or plasma (Citrate, EDTA or Heparin)
Detection Limit:	171 pmol/l at 95% B/Bo
Incubation time:	16-25 hrs / 20 min

For further information on assay characteristics please visit our website [www.bmgrp.com](http://www.bmgrp.com) validation data or contact our customer service by e-mail [info@bmgrp.com](mailto:info@bmgrp.com) or by phone +43/ 1/ 29107-45.

## 10) PRECISION

Intra-Assay: 2 samples of known concentrations were tested 3 times in 1 assay.

Inter-Assay: 2 samples of known concentrations were tested in 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)	763	3,236	Mean (pmol/l)	781	3,199
SD (pmol/l)	43	251	SD (pmol/l)	45	236
CV (%)	6	8	CV (%)	6	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 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.

### 12) PRECAUTIONS

All test components of human source were tested with 3<sup>rd</sup> generation tests 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 950 as preservative. Avoid contact with skin and mucous membrane. Proclin 950 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!!

### 13) LITERATURE

1. Thirty years of the heart as an endocrine organ: physiological role and clinical utility of cardiac natriuretic hormones. Clerico A et al., Am J Physiol Heart Circ Physiol 2011; 301: H12-H20
2. Comparison of Pleural Fluid N-Terminal Pro-Brain Natriuretic Peptide and Brain Natriuretic-32 Peptide Levels. Long AC et al., Chest 2010; 137: 1369-1374
3. N-Terminal Pro-B-Type Natriuretic Peptide as an Indicator of Possible Cardiovascular Disease in Severely Obese Individuals: Comparison with Patients in Different Stages of Heart Failure. Hermann-Arnhof K et al., Clinical Chemistry 2005; 51:138-143
4. Natriuretic Peptides: New Players in Energy Homeostasis. Moro C and Smith RH, Diabetes 2009; 58: 27-26
5. 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., Eur Heart J 2004; 26: 250-256
6. Natriuretic peptides: Markers or modulators of cardiac hypertrophy? Gardner DG, *Trends Endocrinol Metab* 2003; 14: 411-416
7. Neurohormonal risk stratification for sudden death and death owing to progressive heart failure in chronic heart failure. Berger R et al, Eur J Clin Invest 2005; 35, 24-31
8. Natriuretic peptides/cGMP/cGMP-dependent protein kinase cascades promote muscle mitochondrial biogenesis and prevent obesity. Miyashita K et al., Diabetes 2009; 58: 2880-2892
9. Processing of Pro-B-Type Natriuretic Peptide: Furin and Corin as Candidate Convertases. Semenov AG et al., Clin Chem. 2010; 56:1166-1176

# 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 expirácie / Doba expirace



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 / Vegyűk figyelembe a használati utasításban foglaltakat / Postupujte podľa pokynov na použitie / Postupujte dle návodu k použití



Lot-Batch Number / Charge-Chargennummer / Lot-Code du lot / Lotto-Numero di lotto / Lote-Código de lote / Lote-Código do lote / Lot-Partijnummer / Lot-Partijcode / 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 / Vyrobene / Vyrobena



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 / Opbevaars mellem / Förvaras vid / Przechowywać w / Tároljuk ..... között / Skladujcie w rozsahu / Skladujcie w 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

# ***BI-20852W BNP FRAGMENT EIA 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 aluminium bag and mark positions on the protocol sheet.

## ***TEST PROCEDURE:***

- ☐ Add 150 µl ASYBUF (Assay buffer) into all wells.
- ☐ Add 30 µl STD/SAMPLE/CTRL (Standard/Sample/Control) into respective wells, swirl gently.
- ☐ Add 50 µl CONJ (Conjugate) into each well, swirl gently.
- ☐ **Cover tightly and incubate overnight (16-25 hours) at 4°C (2-8°C), in the dark.**
- ☐ Aspirate and wash wells with 300 µl diluted WASHBUF (Wash buffer) five times. Remove remaining buffer by hitting plate against paper towel.
- ☐ Add 200 µl SUB (Substrate) into each well.
- ☐ **Incubate for 20 minutes at room temperature (18-26°C), in the dark.**
- ☐ Add 50 µl STOP (Stop solution) into each well, swirl gently.
- ☐ Read Optical Density at 450 nm with reference 630 nm, if available.