**BIOMEDICA**
I M M U N O A S S A Y S

# NT-proBNP ELISA (Cat.No. SK-1204)

## For the Determination of NT-proBNP (1-76) in Human Samples

## ASSAY CHARACTERISTICS

| Method | Sandwich ELISA, HRP/TMB, 12x8-well strips |
|---|---|
| Antibodies/Standard | Capture antibody: polyclonal anti human NT-proBNP antibody, specifically binding to amino acids 31-57 of proBNP.<br>Detection antibody: polyclonal anti human NT-proBNP antibody, conjugated to peroxidase, specifically binding to amino acids 8-29 of proBNP.<br>Standard material: synthetic human NT-proBNP (1-76). |
| Sample type | Human serum, EDTA plasma |
| Standard range | 0-640 pmol/l *(0, 10, 40, 160, 640)* |
| Conversion factor | 1 pmol/l = 8.475 pg/ml<br>*refers to NT-proBNP (1-76) that is detected by the ELISA* |
| Sample volume | 50 µl / well – direct measurement |
| Sensitivity | LOD (0pmol/l + 3SD): 3.0 pmol/l; LLOQ: 3.3 pmol/l |
| Specificity | This assay recognizes endogenous (natural) and recombinant human NT-proBNP (1-76). |
| Incubation | 3 h /30 min – room temperature |

## Typical standard curve of Biomedica NT-proBNP ELISA:
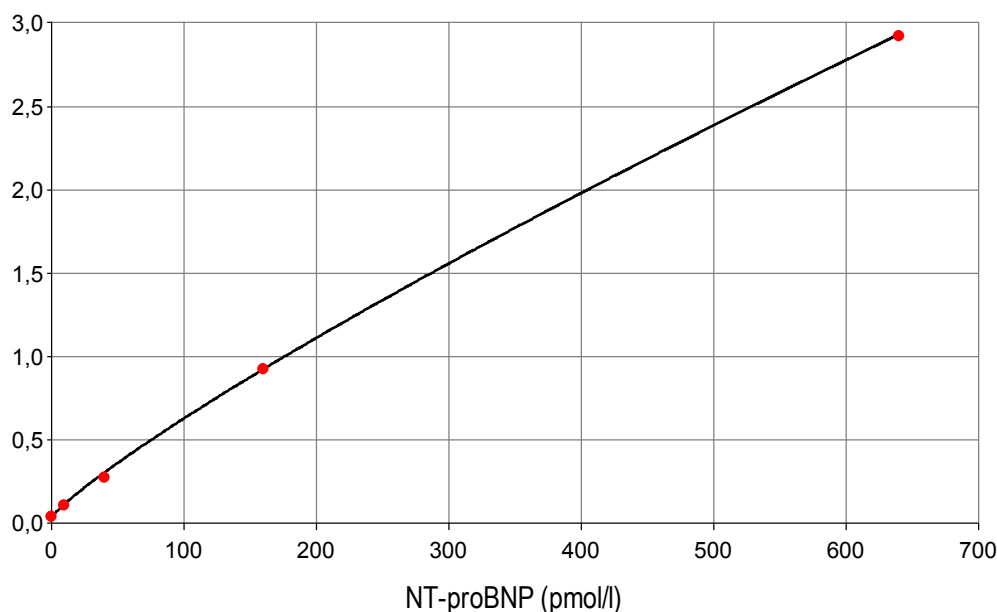
**Serum values from apparently healthy donors:**

| Serum (n=70) | NT-proBNP (pmol/l) |
|---|---|
| **Median** | **5.8** |
| Mean | 9.8 |
| Maximum | 63.9 |
| Minimum | 1.1 |
| Percentil 95% | 32.5 |
| Percentil 5% | 2.0 |

**EDTA plasma values from apparently healthy donors:**

| EDTA plasma (n=28) | NT-proBNP (pmol/l) |
|---|---|
| **Median** | **5.6** |
| Mean | 9.5 |
| Maximum | 32.0 |
| Minimum | 0.6 |
| Percentil 95% | 27.9 |
| Percentil 5% | 0.8 |

**Serum values from unselected hospital panel:**

| Serum (n=117) | NT-proBNP (pmol/l) |
|---|---|
| **Median** | **46.5** |
| Mean | 84.5 |
| Maximum | 621.1 |
| Minimum | 0.0 |
| Percentil 95% | 22.1 |
| Percentil 5% | 0.8 |

**EDTA plasma values from unselected hospital panel:**

| EDTA plasma (n=40) | NT-proBNP (pmol/l) |
|---|---|
| **Median** | **28.6** |
| Mean | 57.9 |
| Maximum | 489.2 |
| Minimum | 0.3 |
| Percentil 95% | 157.7 |
| Percentil 5% | 1.2 |

**Serum values of a heart failure panel NYHA classification II-IV measured with the Biomedica NT-proBNP ELISA (#SK-1204)**

| Serum (n=63) | NT-proBNP (pmol/l) |
|---|---|
| mean | 54.0 |
| max | 399.3 |
| min | 1.1 |

## NYHA classification subgroups:
measured with the Biomedica NT-proBNP ELISA (#SK-1204)

Data on serum values of a heart failure panel **NYHA classification II**

| Serum (n=41) | NT-proBNP (pmol/l) |
|---|---|
| mean | 25.5 |
| max | 75.6 |
| min | 1.1 |

Data on serum values of a heart failure panel **NYHA classification III**

| Serum (n=14) | NT-proBNP (pmol/l) |
|---|---|
| mean | 63.2 |
| max | 264.3 |
| min | 9.2 |

Data on serum values of a heart failure panel **NYHA classification IV**

| Serum (n=7) | NT-proBNP (pmol/l) |
|---|---|
| mean | 166.8 |
| max | 399.3 |
| min | 84.1 |

## MATRIX COMPARISON

### Correlation of serum and plasma samples from apparently healthy individuals



Plasma/Serum Matrix Comparison of an Apparently Healthy Cohort

$y = 0,9359x$
$R^2 = 0,9842$
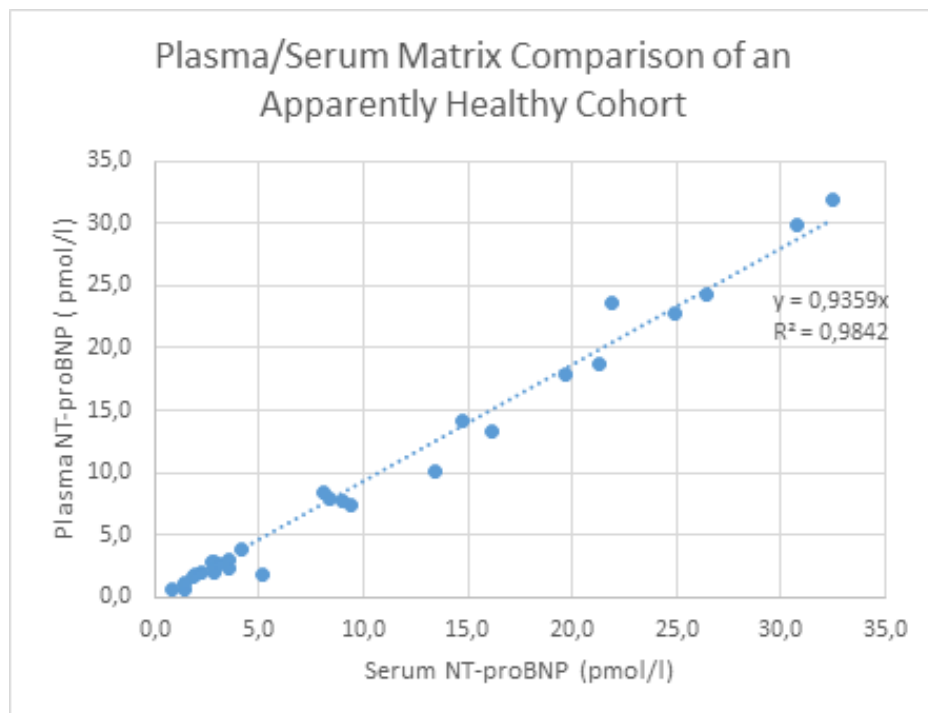
## PERFORMANCE CHARACTERISTICS

### Spike Recovery:

**The mean recovery of rec. NT-proBNP in serum samples is 99 and 108%.**

Experiment:
Recovery of spiked samples was tested by adding 2 concentrations of human recombinant NT-proBNP (1-76) to 4 different human serum samples.

Data showing spike/recovery of human serum samples:

| Matrix | | Serum | | | S/R (%) | |
|---|---|---|---|---|---|---|
| Spike (pmol/l) | | 0 | 80 | 320 | 80 | 320 |
| | #1 | 1.6 | 88.5 | 347.2 | 109 | 108 |
| | #2 | 0.1 | 74.2 | 360.2 | 93 | 113 |
| Sample ID | #3 | 1.7 | 80.9 | 338.7 | 99 | 105 |
| | #4 | 4.4 | 81.6 | 346.5 | 96 | 107 |
| | | | | Mean (%) | 99 | 108 |

**The mean recovery of rec. NT-proBNP in EDTA plasma samples is 93 and 94%.**

Experiment:
Recovery of spiked samples was tested by adding 2 concentrations of human recombinant NT-proBNP (1-76) to 4 different human EDTA plasma samples.

Data showing spike/recovery of human EDTA plasma samples:

| Matrix | | EDTA plasma | | | S/R (%) | |
|---|---|---|---|---|---|---|
| Spike (pmol/l) | | 0 | 80 | 320 | 80 | 320 |
| | #1 | 0.8 | 73.4 | 276.8 | 92 | 87 |
| | #2 | 7.7 | 72.8 | 279.0 | 91 | 87 |
| Sample ID | #3 | 9.3 | 77.2 | 324.1 | 97 | 101 |
| | #4 | 19.1 | 74.9 | 309.5 | 94 | 97 |
| | | | | Mean (%) | 94 | 93 |

### Dilution Linearity:

**Serum (n=3):**    **112-119%** (spiked with recombinant NT-proBNP)
                    **79-86%** (endogenous NT-proBNP)

Experiment 1:
Dilution linearity was assessed by spiking serum samples with recombinant NT-proBNP (1-76) (320 pmol/l) and by diluting the samples with STD1 (serum matrix containing 0 pmol/l NT-proBNP, provided in the kit):

Data showing the dilution of <u>recombinant NT-proBNP (1-76) in serum samples</u>:

| Serum Sample ID | Reference NT-proBNP (pmol/l) | Spiked NT-proBNP (pmol/l) | Dilution 1+1 | |
|---|---|---|---|---|
| | | | NT-proBNP (pmol/l) | R (%) |
| #1 | 1.7 | 338.7 | 202.2 | 119 |
| #2 | 0.1 | 360.2 | 202.4 | 112 |
| #3 | 8.0 | 278.5 | 165.7 | 119 |
| | | | **Mean (%)** | **117** |

Experiment 2:
Dilution linearity was assessed by diluting serum samples containing endogenous NT-proBNP with STD1 (serum matrix containing 0 pmol/l NT-proBNP, provided in the kit).

Data showing the dilution of <u>endogenous NT-proBNP (1-76) in serum samples</u>:

| Serum Sample ID | Reference NT-proBNP (pmol/l) | Dilution 1+1 | |
|---|---|---|---|
| | | NT-proBNP (pmol/l) | R (%) |
| #1 | 44.2 | 17.4 | 79 |
| #2 | 51.3 | 20.4 | 80 |
| #3 | 61.7 | 26.5 | 86 |
| | | **Mean (%)** | **82** |

**EDTA plasma (n=2):**   **83-84%** (spiked with recombinant NT-proBNP)
    **80-85%** (endogenous NT-proBNP)

Experiment 1:
Dilution linearity was assessed by spiking EDTA plasma samples 1+1 with recombinant NT-proBNP (1-76) (640 pmol/l) and by diluting the samples with STD1 (serum matrix containing 0 pmol/l NT-proBNP, provided in the kit):

Data showing the dilution of <u>recombinant NT-proBNP (1-76) in EDTA plasma samples</u>:

| EDTA plasma Sample ID | Reference NT-proBNP (pmol/l) | Spiked NT-proBNP (pmol/l) | Dilution 1+1 | |
|---|---|---|---|---|
| | | | NT-proBNP (pmol/l) | R (%) |
| #1 | 36 | 347 | 145 | 84 |
| #2 | 0.8 | 277 | 115 | 83 |
| | | | **Mean (%)** | **84** |

Experiment 2:
Dilution linearity was assessed by diluting EDTA plasma samples containing endogenous NT-proBNP with STD1 (serum matrix containing 0 pmol/l NT-proBNP, provided in the kit).

BIOMEDICA
IMMUNOASSAYS

Data showing the dilution of <u>endogenous NT-proBNP (1-76) in EDTA plasma samples</u>:

| EDTA plasma Sample ID | Reference NT-proBNP (pmol/l) | Dilution 1+1 NT-proBNP (pmol/l) | R (%) |
|---|---|---|---|
| #1 | 269 | 114 | 85 |
| #2 | 634 | 263 | 83 |
| | | **Mean (%)** | **84** |

## Intra-assay precision & Inter-assay precision:

## Intra-assay (n=3) ≤ 4%, Inter-assay (n=8) ≤ 7%

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.

Data showing intra-assay and inter-assay precision:

| 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 |

## The limit of quantification (LOQ):

The LOQ is defined as the mean value of the back calculated concentration plus 3 times the standard deviation. The LOQ of the NT-proBNP ELISA is 3 pmol/l.

## The lower limit of quantification (LLOQ):

The lower limit of quantification is defined as the accuracy of the back calculated concentrations and shall not exceed ±25% (acc. to ICH (Ref. 1)).
For the NT-proBNP ELISA the LLOQ was determined as 3.3 pmol/l.

## Specificty:
This assay recognizes endogenous (natural) and recombinant human NT-proBNP (1-76).

## Calibration:
This immunoassay is calibrated against recombinant human NT-proBNP (1-76) peptide.

**BIOMEDICA**
IMMUNOASSAYS

## SAMPLE CHARACTERISTICS

### Sample stability:

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 EDTA plasma or serum samples and store them at -25°C or lower.
Samples can be subjected to 3 freeze-thaw cycles.
Serum and EDTA samples can be stored for at least 2 years at -80°C.

### Freeze/thaw of samples containing endogenous NT-proBNP

The mean recovery of serum and EDTA plasma sample concentrations stressed by 3 F/T cycles is 94%. The mean CV of sample concentrations (not stressed and stressed up to 3 times by freeze-thaw cycles) is 4%.

NT-proBNP concentrations of samples after 3 freeze-thaw cycles:

| Sample ID | NT-proBNP [pmol/l] | | | | | CV (%) | R (%) 3 F/T vs ref |
|---|---|---|---|---|---|---|---|
| # of F/T cycles | Ref | 1 | 2 | 3 | Mean | | |
| Serum1 | 70 | 67 | 67 | 69 | 68 | 1 | 98 |
| Serum2 | 15 | 13 | 15 | 14 | 14 | 5 | 98 |
| Serum3 | 215 | 185 | 191 | 188 | 194 | 6 | 87 |
| Serum4 | 141 | 129 | 129 | 126 | 131 | 4 | 90 |
| Serum5 | 49 | 47 | 48 | 47 | 48 | 2 | 96 |
| Plasma1 | 203 | 191 | 199 | 187 | 195 | 3 | 92 |
| Plasma2 | 282 | 257 | 271 | 248 | 264 | 5 | 88 |
| Plasma3 | 68 | 64 | 79 | 69 | 70 | 8 | 101 |
| | | | | | **Mean (%)** | **4** | **94** |



F/T of serum and plasma samples
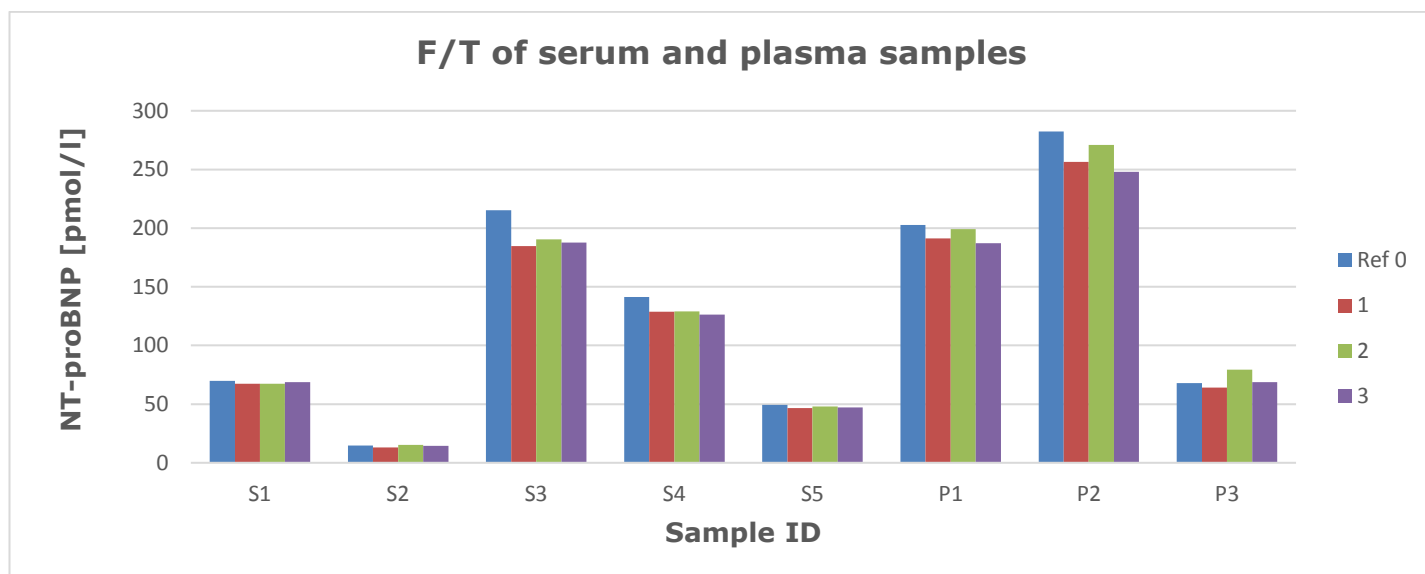
## Validation

The assay is fully validated according to ICH Q2 (R1), Ref. (1).

## References

(1) CPMP/ICH/381/95 - ICH Topic Q2 (R1) „Validation of Analytical Procedures: Text and Methodology" including:

ICH Q2A "Text on Validation of Analytical Procedures"

ICH Q2B "Validation of Analytical Procedures: Methodology"

## Available on our homepage

Enzyme immunoassay for the quantitative determination of NT-proBNP (1-76)  in human serum.

Package Insert, MSDS, Information Folder, and References:
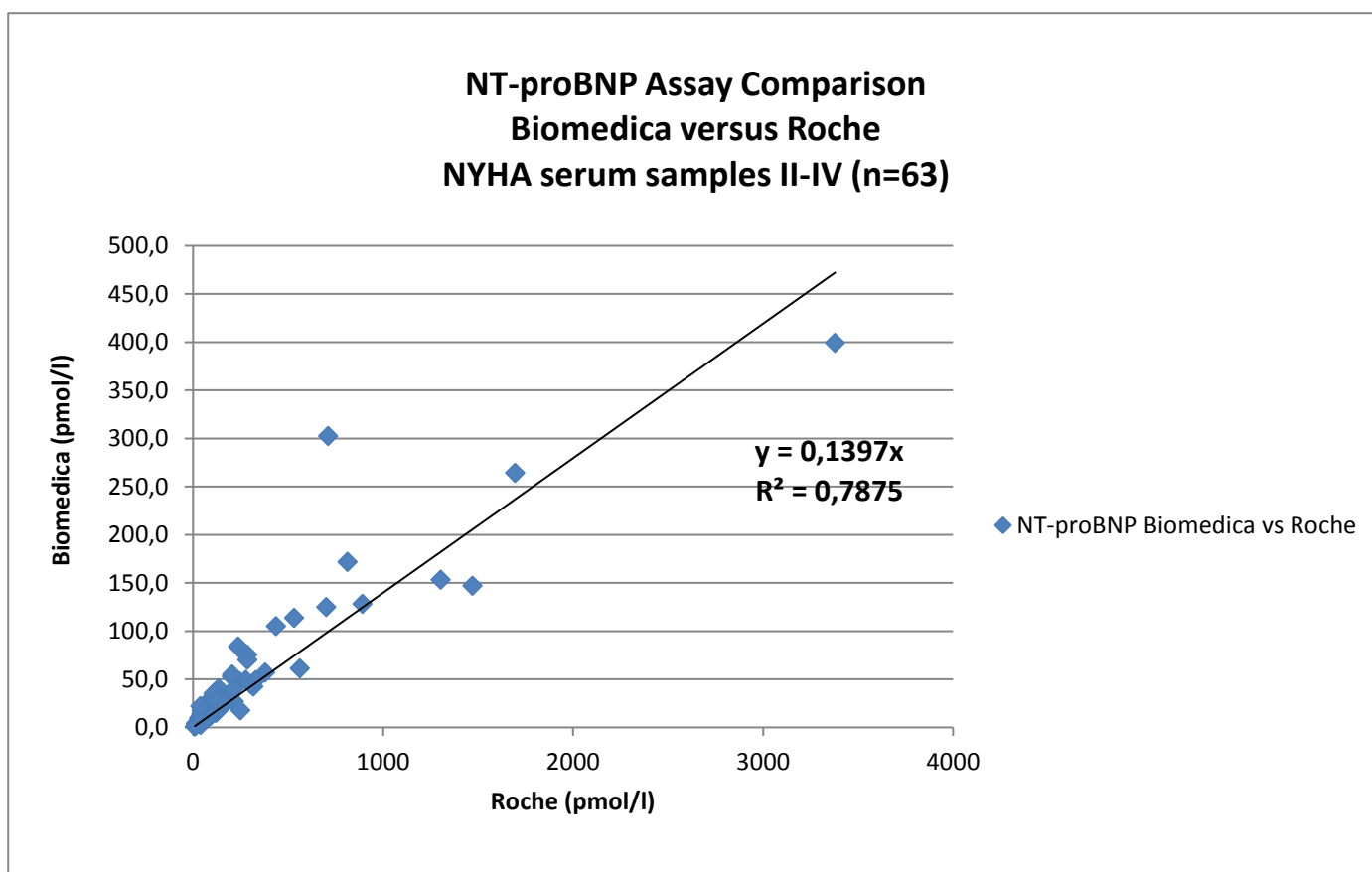
http://www.bmgrp.com/products/cardiovascular/nt-probnp-elisa/

# NT-proBNP ASSAY COMPARISON

## Comparison between NT-proBNP Assays:
## Biomedica SK-1204 versus Roche Elecys 2nd generation Assay

Data on serum values of a heart failure panel NYHA classification II-IV measured with the Biomedica NT-proBNP ELISA (#SK-1204):

| n | 63 |
|---|---|
| mean | 54.0 pmol/l |
| max | 399.3 pmol/l |
| min | 1.1 pmol/l |



**NT-proBNP Assay Comparison
Biomedica versus Roche
NYHA serum samples II-IV (n=63)**

$y = 0,1397x$
$R^2 = 0,7875$

◆ NT-proBNP Biomedica vs Roche
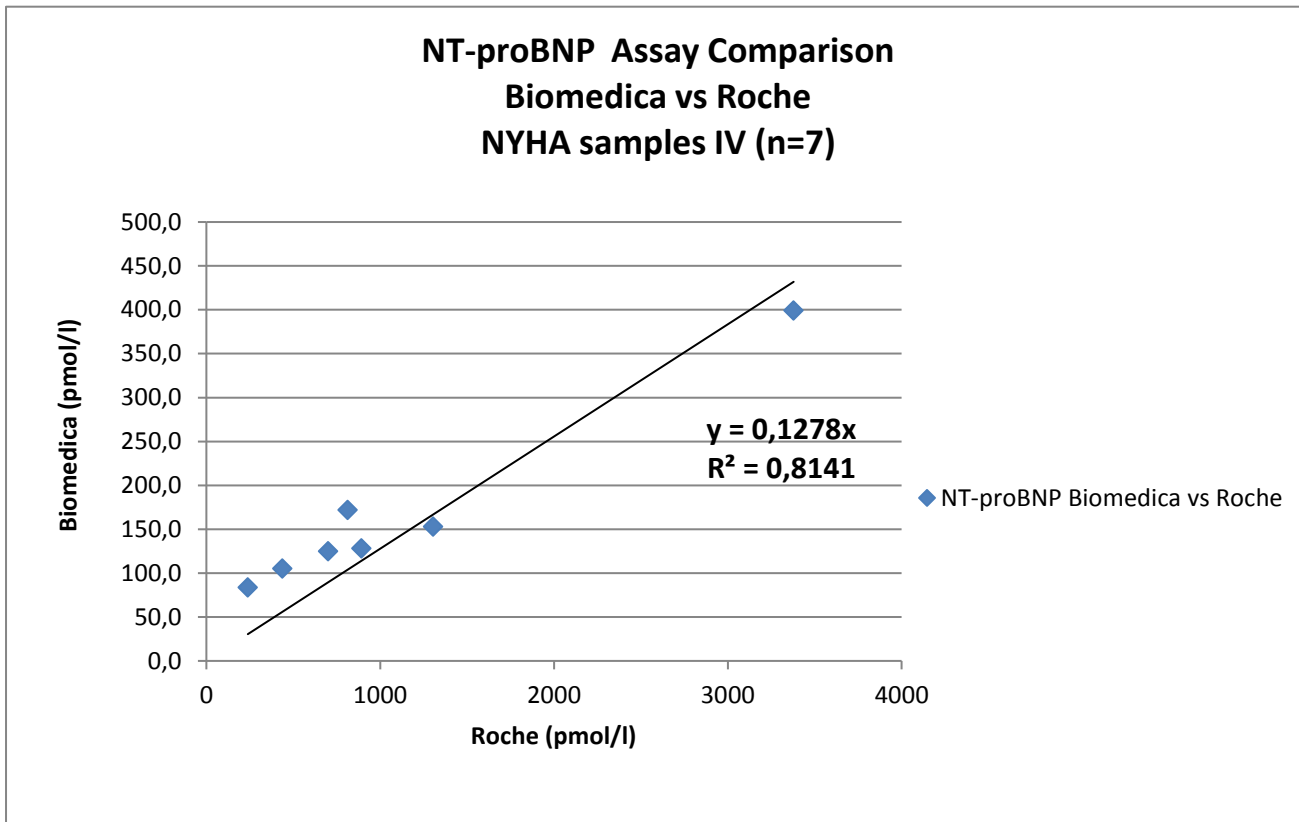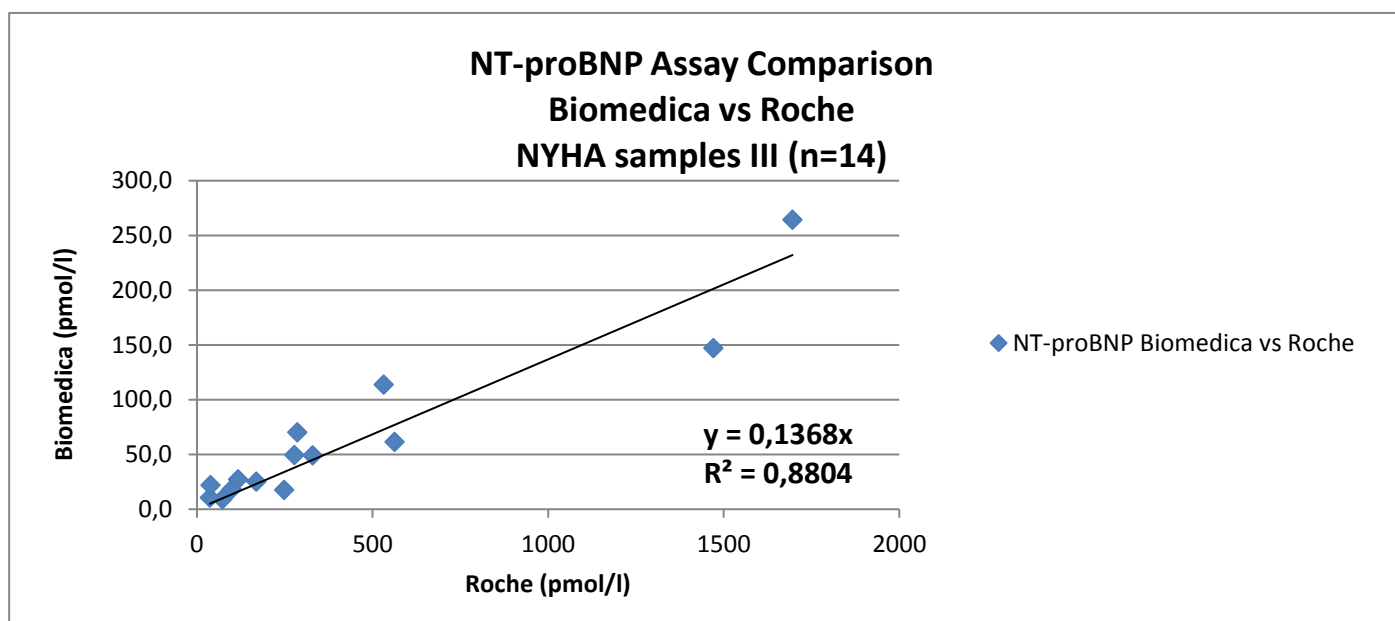
Data on serum values of a heart failure panel NYHA classification IV measured with the Biomedica NT-proBNP ELISA (#SK-1204)

| n | 7 |
|---|---|
| mean | 166.8 pmol/l |
| max | 399.3 pmol/l |
| min | 84.1 pmol/l |

## NT-proBNP  Assay Comparison
### Biomedica vs Roche
### NYHA samples IV (n=7)



$y = 0.1278x$
$R^2 = 0.8141$

Data on serum values of a heart failure panel NYHA classification III measured with the Biomedica NT-proBNP ELISA (#SK-1204)

| n | 14 |
|---|---|
| mean | 63.2 pmol/l |
| max | 264.3 pmol/l |
| min | 9.2 pmol/l |

## NT-proBNP Assay Comparison
### Biomedica vs Roche
### NYHA samples III (n=14)



$y = 0.1368x$
$R^2 = 0.8804$

Data on serum values of a heart failure panel NYHA classification II measured with the Biomedica NT-proBNP ELISA (#SK-1204)

| n | 41 |
|------|-------------|
| mean | 25,5 pmol/l |
| max | 75,6 pmol/l |
| min | 1,1 pmol/l |



**NT-proBNP Assay Comparison
Biomedica vs Roche
NYHA samples II (n=41)**

y = 0,1963x
R² = 0,7505