

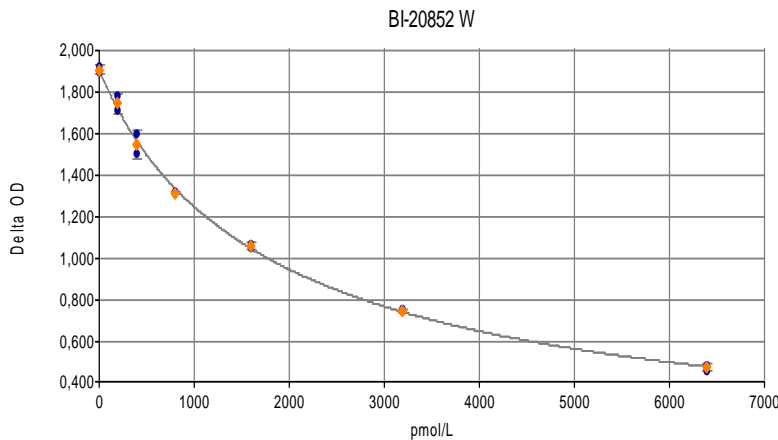
## BNP Fragment EIA (Cat.No. BI-20852W)

### For the Determination of BNP Fragment in Human Samples

#### ASSAY CHARACTERISTICS

|                               |   |
|-------------------------------|---|
| <b>Method</b>                 | Competitive Enzyme Immunoassay, HRP/TMB.<br>Microtiter plates are coated with a polyclonal anti BNP Fragment (8-29) antibody. |
| <b>Sample type</b>            | Serum, plasma   |
| <b>Standard range</b>         | 0 – 6,400 pmol/l (0 / 400 / 800 / 1,600 / 3,200 / 6,400 pmol/l)   |
| <b>Conversion factor</b>      | 8.4 pg/ml = 1 pmol/l  |
| <b>Sample volume</b>          | 30 µl   |
| <b>Detection limit</b>        | 171 pmol/L at 95% B/Bo  |
| <b>Incubation time, temp.</b> | Overnight +4°C / 20 min room temperature  |
| <b>Cross reactivity</b>       | The assay does not cross react with rat or mouse samples.   |

#### Typical Standard Curve of BI-20852W



#### Serum values from apparently healthy individuals

|                 |              |
|-----------------|--------------|
|                 | Serum (n=76) |
| Median (pmol/l) | 392          |

## **PERFORMANCE CHARACTERISTICS**

### **Spike Recovery**

Analyte: recombinant BNP precursor containing fragments of AA 8-29.

The mean recovery of recombinant BNP Fragment in serum is 100%.

The mean recovery of recombinant BNP Fragment in citrate plasma is 108%.

Experiment: Recovery of spiked samples was tested by adding two different concentrations of recombinant BNP Fragment (533 and 2,133 pmol/l) to four different human serum samples.

Data showing spike/recovery of serum samples:

| matrix                              |    | serum |       |       | S/R         |            |
|-------------------------------------|----|-------|-------|-------|-------------|------------|
| spike of rec. BNP Fragment (pmol/l) |    | 0     | 533   | 2,133 | 533         | 2,133      |
| sample ID                           | #1 | 1,241 | 1,709 | 3,128 | 96%         | 93%        |
|                                     | #2 | 2,069 | 2,194 | 3,258 | 84%         | 78%        |
|                                     | #3 | 66    | 746   | 2,091 | 125%        | 95%        |
|                                     | #4 | 0     | 645   | 2,403 | 121%        | 113%       |
| <b>Mean (%)</b>                     |    |       |       |       | <b>107%</b> | <b>94%</b> |

Experiment: Recovery of spiked samples was tested by adding 4,869 pmol/l of recombinant BNP Fragment to nine different human citrate plasma samples.

Data showing spike/recovery of citrate plasma samples:

| matrix                              |    | Citrate plasma |       | S/R         |
|-------------------------------------|----|----------------|-------|-------------|
| spike of rec. BNP Fragment (pmol/l) |    | 0              | 4,869 | 4,869       |
| sample ID                           | #1 | 221            | 4,816 | 95%         |
|                                     | #2 | 528            | 6,061 | 112%        |
|                                     | #3 | 312            | 6,057 | 117%        |
|                                     | #4 | 512            | 6,052 | 112%        |
|                                     | #5 | 462            | 5,911 | 111%        |
|                                     | #6 | 448            | 5,613 | 106%        |
|                                     | #7 | 285            | 5,768 | 112%        |
|                                     | #8 | 485            | 6,339 | 118%        |
|                                     | #9 | 1,021          | 5,319 | 90%         |
| <b>Mean (%)</b>                     |    |                |       | <b>108%</b> |

### Dilution Linearity

Analyte: recombinant BNP precursor containing fragments of AA 8-29.

The mean dilution linearity of recombinant BNP Fragment protein in serum samples (n=7) is 105% for a 1+1 dilution and 131% for a 1+3 dilution.

Data showing the dilution of recombinant BNP Fragment:

|                 | reference | spike of rec. BNP Fragment | dilution 1+1 |             | dilution 1+3 |             |
|-----------------|-----------|----------------------------|--------------|-------------|--------------|-------------|
| sample ID       | pmol/l    | 1,217 pmol/l               | pmol/l       | R           | pmol/l       | R           |
| #1              | 14        | 1,384                      | 661          | 96%         | 338          | 98%         |
| #2              | 418       | 2,388                      | 1,236        | 104%        | 782          | 131%        |
| #3              | 352       | 2,214                      | 1,216        | 110%        | 779          | 141%        |
| #4              | 1,179     | 3,387                      | 1,588        | 94%         | 968          | 114%        |
| #5              | 1,268     | 2,642                      | 1,464        | 111%        | 1016         | 154%        |
| #6              | 955       | 2,482                      | 1,360        | 110%        | 886          | 143%        |
| #7              | 816       | 2,451                      | 1,382        | 113%        | 841          | 137%        |
| <b>Mean (%)</b> |           |                            |              | <b>105%</b> |              | <b>131%</b> |

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

The precision data for the BNP Fragment Assay: Intra-assay < 8%, Inter-assay < 7%

#### Experiment:

Intra-assay: 2 samples of known concentrations were tested 3 times in 1 assay by one 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)     | 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        |

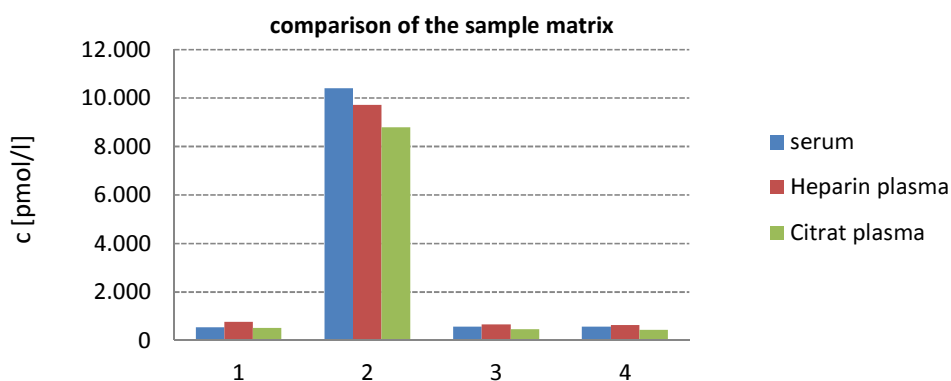
## SAMPLE CHARACTERISTICS

### Effect of sample matrix

Measurement of BNP Fragment in 3 different sample matrices from 4 samples showed a mean CV of 17%. All 3 matrices can be tested in the assay.

Data showing the effect of the sample matrix:

| Sample ID       | pmol/l |                |                |       | CV         |
|-----------------|--------|----------------|----------------|-------|------------|
|                 | serum  | Heparin plasma | Citrate plasma | Mean  |            |
| #1              | 545    | 770            | 523            | 613   | 22%        |
| #2              | 10,408 | 9,708          | 8,792          | 9,636 | 8%         |
| #3              | 577    | 664            | 462            | 567   | 18%        |
| #4              | 568    | 640            | 435            | 547   | 19%        |
| <b>Mean (%)</b> |        |                |                |       | <b>17%</b> |



Comparison of the BNP Fragment content of four donors in three different sample matrices

### Stability of samples

BNP fragments are stable in whole blood, serum or plasma for several hours at room temperature or +4°C (2-8°C). Nevertheless we recommend separating 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). Aliquot the acquired plasma or 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. Serum samples can be stored for ≥ 2 years at -80°C.

Experiment: Storage of serum samples containing endogenous BNP Fragment overnight at room temperature.

| Sample ID       | c [pmol/l] |         | CV  | R           |
|-----------------|------------|---------|-----|-------------|
|                 | fresh      | o.n. RT |     |             |
| 1               | 803        | 732     | 10% | 91%         |
| 2               | 1,247      | 1,392   | 12% | 112%        |
| 3               | 2,480      | 2,477   | 0%  | 100%        |
| <b>Mean (%)</b> |            |         |     | <b>101%</b> |

The recovery of human serum samples after an overnight storage at room temperature is 101%.

**Data from human serum samples of a panel of blood donors**

76 human serum samples of a panel of blood donors were tested in the BNP Fragment EIA, cat# BI-20852W.

| Sample ID | c [pmol/l] | sample no. | c [pmol/l] | sample no. | c [pmol/l] |
|-----------|------------|------------|------------|------------|------------|
| #1        | 166        | #26        | 492        | #51        | 359        |
| #2        | 408        | #27        | 420        | #52        | 192        |
| #3        | 576        | #28        | 416        | #53        | 341        |
| #4        | 516        | #29        | 614        | #54        | 548        |
| #5        | 351        | #30        | 271        | #55        | 342        |
| #6        | 548        | #31        | 335        | #56        | 244        |
| #7        | 348        | #32        | 327        | #57        | 398        |
| #8        | 348        | #33        | 428        | #58        | 317        |
| #9        | 250        | #34        | 421        | #59        | 253        |
| #10       | 267        | #35        | 598        | #60        | 467        |
| #11       | 270        | #36        | 424        | #61        | 611        |
| #12       | 250        | #37        | 337        | #62        | 769        |
| #13       | 895        | #38        | 1,172      | #63        | 269        |
| #14       | 789        | #39        | 348        | #64        | 243        |
| #15       | 407        | #40        | 383        | #65        | 150        |
| #16       | 270        | #41        | 321        | #66        | 300        |
| #17       | 162        | #42        | 415        | #67        | 244        |
| #18       | 341        | #43        | 523        | #68        | 1,631      |
| #19       | 383        | #44        | 431        | #69        | 431        |
| #20       | 265        | #45        | 453        | #70        | 462        |
| #21       | 364        | #46        | 1,102      | #71        | 784        |
| #22       | 704        | #47        | 455        | #72        | 503        |
| #23       | 243        | #48        | 321        | #73        | 1,045      |
| #24       | 627        | #49        | 578        | #74        | 313        |
| #25       | 287        | #50        | 385        | #75        | 629        |
|           |            |            |            | #76        | 606        |

|                                      |            |
|--------------------------------------|------------|
| n =                                  | 76         |
| mean pmol/l                          | 453        |
| <b>median pmol/l</b>                 | <b>392</b> |
| min pmol/l                           | 150        |
| max pmol/l                           | 1,631      |
| 90 <sup>th</sup> percentile [pmol/l] | 737        |

Values from 76 apparently healthy individuals in a panel of blood donors (serum) showed a median concentration of BNP Fragment of 392 pmol/l.

### Data from serum samples of a dialysis panel

Panel 1: 16 human serum samples of a dialysis panel deriving from a hospital lab were tested in the BNP Fragment EIA, cat# BI-20852W.

Calculation of concentrations of 16 human serum samples from a dialysis panel 1 deriving from a hospital lab:

| Sample ID | c [pmol/l] |
|-----------|------------|
| #1        | 3,200      |
| #2        | 6,081      |
| #3        | 2,280      |
| #4        | >6,400     |
| #5        | 3,070      |
| #6        | >6,400     |
| #7        | 4,178      |
| #8        | >6,400     |
| #9        | 3,863      |
| #10       | 4,036      |
| #11       | 2,806      |
| #12       | 4,142      |
| #13       | 3,653      |
| #14       | 2,374      |
| #15       | 2,893      |
| #16       | >6,400     |

|                      |        |
|----------------------|--------|
| <b>median pmol/l</b> | 3,426  |
| min pmol/l           | 2,280  |
| max pmol/l           | >6,400 |

Values from 16 human serum samples from a dialysis panel showed a median concentration of BNP Fragment of 3,426 pmol/l.

Panel 2: 16 human serum samples of a dialysis panel deriving from a hospital lab were tested in the BNP Fragment EIA, cat# BI-20852W.

Calculation of concentrations of 16 human serum samples from a dialysis panel 2 deriving from a hospital lab:

| Sample ID | c [pmol/l] |
|-----------|------------|
| #1        | 3,375      |
| #2        | 6,120      |
| #3        | >6,400     |
| #4        | 1,197      |
| #5        | 4,331      |
| #6        | 5,146      |
| #7        | 781        |
| #8        | 698        |
| #9        | 3,321      |
| #10       | >6,400     |
| #11       | 1,386      |
| #12       | 3,549      |
| #13       | 4,370      |
| #14       | 2,662      |
| #15       | 2,072      |
| #16       | 1,404      |

|               |        |
|---------------|--------|
| median pmol/l | 3,348  |
| min pmol/l    | 698    |
| max pmol/l    | >6,400 |

Values from 16 human serum samples from a dialysis panel showed a median concentration of BNP Fragment of 3,348 pmol/l.

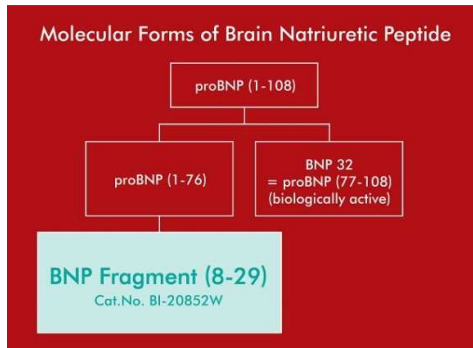
### **Validation**

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” and ICH Q2B “Validation of Analytical Procedures: Methodology”

### Additional information



### Did you know?

#### Biomedica's BNP Fragment Assay is:

- **Specific** - to human NT-proBNP 8-29.
- **Stable** - epitope region
- **Optimal** - immunodetection outside of the glycosylated region

### Available on our Website

#### Package insert BNP Fragment EIA, Cat.No. BI-20852W

Enzyme immunoassay for the quantitative determination of BNP Fragment in human serum, citrate plasma, EDTA plasma or heparin plasma

[http://www.bmgrp.com/fileadmin/user\\_upload/immunoassays/BI-20852W\\_BNP\\_Fragment\\_120613.pdf](http://www.bmgrp.com/fileadmin/user_upload/immunoassays/BI-20852W_BNP_Fragment_120613.pdf)

#### Information folder on natriuretic peptides

[http://www.bmgrp.com/fileadmin/user\\_upload/immunoassays/BI-20852\\_Biomedica\\_Natriuretische\\_Peptide\\_19032013.pdf](http://www.bmgrp.com/fileadmin/user_upload/immunoassays/BI-20852_Biomedica_Natriuretische_Peptide_19032013.pdf)

#### Material Safety Data Sheet

[http://www.bmgrp.com/fileadmin/user\\_upload/immunoassays/BI-20852\\_MSDS\\_BNP\\_Fragment\\_W\\_130108.pdf](http://www.bmgrp.com/fileadmin/user_upload/immunoassays/BI-20852_MSDS_BNP_Fragment_W_130108.pdf)