proANP (1-98) ELISA, Cat.No. BI-20892

For the quantitative determination of proANP (1-98) in human samples

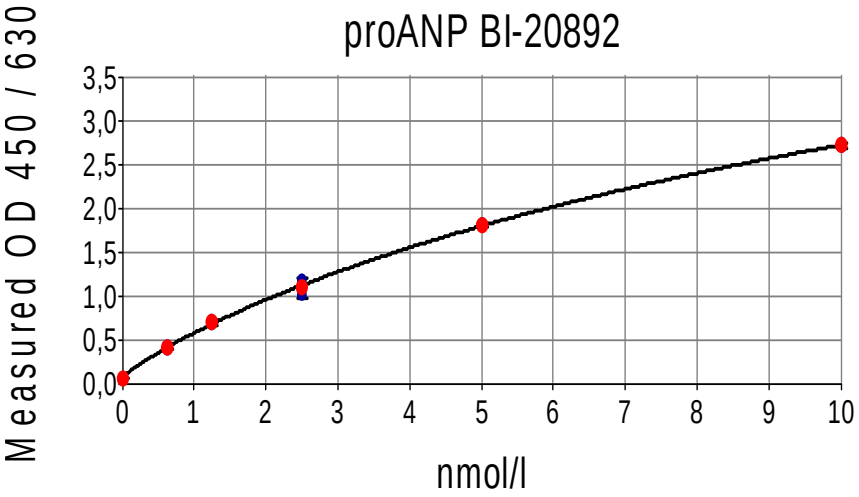
ASSAY CHARACTERISTICS

Method	Sandwich ELISA, 96-well strip plate, HRP/TMB
Sample type	Serum, EDTA-plasma, heparin plasma, CC supernatants, urine
Standard range	0-10 nmol/l *(0/0.63/1.25/2.5/5/10 nmol/l)*
Conversion factor	The MW of the measured proANP (1-98) is: 12,700 Da. The conversion factor is: 1 nmol/l = 12.7 ng/ml or 1 ng/ml = 0.079 nmol/l
Sample volume	10 µl per well
Detection limit	(0 nmol/l + 3 SD): 0.050 nmol/l
Incubation time, temp.	3 h / 30 min, room temperature
Cross reactivity	proANP (1-30) <1%, proANP (31-67) <1%, proANP (79-98) <1%, alpha ANP (99-126)<1%, proBNP (8-29) <1%, proBNP (32-57) <1%, proCNP (1-19) <1%, proCNP (30-50) <1%, proCNP (51-97) <1%. The assay also detects mouse and rat proANP (1-98).

Kit specific components

- Coating antibody: plate coated with sheep anti human proANP directed against the N-terminal region of proANP (1-98)
- Detection antibody: HRP-labeled sheep anti human proANP (1-98) directed against the C-terminal region of proANP (1-98), ready to use
- Standard material: recombinant human proANP (1-98) in human plasma, concentration: 0-10 nmol/l , lyophilized

Typical standard curve:



Values from apparently healthy individuals:

Sample	EDTA-plasma (n=53)
Median (nmol/l)	1.45

It is recommended that each laboratory establishes its own reference range for the samples under investigation.

proANP (1-98) values from an unselected hospital panel

Sample	EDTA-plasma (n=40)
Mean (nmol/l)	3.89
Median (nmol/l)	3.09
Min (nmol/l)	0.63
Max (nmol/l)	10.50

PERFORMANCE CHARACTERISTICS

Spike/Recovery:

Recovery was tested by adding 2 different concentrations of recombinant proANP (1-98) to human EDTA-plasma samples (n=4).

Sample	Spike proANP (nmol/l)	Mean recovery (%)
EDTA-plasma (n=4)	0.81	87
EDTA-plasma (n=4)	4.05	89

Dilution linearity:

3 freshly collected EDTA-plasma samples were diluted with ASYBUF (assay buffer).

Sample matrix	Dilution	Mean recovery (%)
EDTA-plasma (n=3)	1+1	90
	1+3	87
	1+7	79

Intra-assay precision & Inter-assay precision:

Intra-assay: 2 samples of known concentrations were tested 5 times within 1 kit lot by 1 operator.
Inter-assay: 2 samples of known concentrations were tested 3 times within 3 different kit assay lots by two different operators.

Intra-assay (n=5)	Sample 1	Sample 2	Inter-assay (n=3)	Sample 1	Sample 2
Mean (nmol/l)	1.07	7.58	Mean (nmol/l)	1.11	7.24
SD (nmol/l)	0.05	0.16	SD (nmol/l)	0.10	0.37
CV (%)	5	2	CV (%)	9	5

SAMPLE CHARACTERISTICS

Sample preparation:

This assay is suitable for the use of EDTA- or heparinised plasma, urine or cell culture supernatants and serum as follows:

ProANP in freshly collected blood samples is stable for at least 2.5 hours at room temperature (18-26°C). Nevertheless we recommend plasma separation by centrifugation as soon as possible described as below:
Collect venous blood samples by using standardized blood collection tubes for serum. Allow samples to clot for 30 minutes at room temperature before performing serum separation by centrifugation, e.g. 20 min at 2000 x g, preferably at 4°C (2-8°C). Measure the acquired samples immediately or aliquot samples in polypropylene tubes and store at -25°C or lower. Avoid more than three freeze-thaw cycles.
Lipemic or hemolyzed samples may give erroneous results. Urine or cell culture supernatants are used neat, without any further treatment. Samples should be mixed well before assaying. We recommend duplicates for all values. If samples read higher than the top standard, we recommend to dilute with ASYBUF (assay buffer) provided with the kit – e.g.: 1+4 and 1+9 and re-measure the samples.

The assay can also be used with serum samples under the following conditions:
Serum separation is performed within 1hr after blood collection. The samples must be tested immediately after separation or must be stored at -25°C/-70°C, not subjected to more than 2 freeze/thaw cycles. This is due to the lower stability of proANP (1-98) in serum compared to EDTA plasma.

The effect of the sample matrix on proANP (1-98) was investigated; no influence of the sample matrix was observed between EDTA- and Heparin Plasma.

Effect of freezing/thawing:

The mean recovery of proANP (1-98) concentration in 6 freshly collected human EDTA-plasma samples after 3 freeze-thaw (F/T) cycles is 88%. Thus samples can be frozen at least 3 times. After 3 F/T cycles a maximum loss of immune reactivity of 20% was monitored. No more than 3 F/T cycles are recommended.

Experiment:
6 freshly collected EDTA-plasma samples have been aliquoted, stored frozen and stressed by additional F/T cycles (frozen by storing them for 60 min at -25°C and the thawing process was at room temperature (18-26°C) for 60 min). The stressed samples were compared.

Sample ID	F/T Cycle #1 c (nmol/l)	F/T Cycle #2 c (nmol/l)	F/T Cycle #3 c (nmol/l)	Signal retained
#1	1.02	0.96	0.82	80%
#2	5.44	5.42	4.75	87%
#3	0.66	0.61	0.59	90%
#4	1.54	1.52	1.23	80%
#5	3.97	3.46	3.42	86%

Available on our Website

Package insert
http://www.bmgrp.com/fileadmin/user_upload_immunoassays/downloads/IFU/BI-20892_proANP_ELISA_IFU.pdf

Material Safety Data Sheet
http://www.bmgrp.com/fileadmin/user_upload_immunoassays/downloads/MSDS/BI-20892_proANP_ELISA_MSDS.pdf

Reference List
http://www.bmgrp.com/fileadmin/user_upload_immunoassays/downloads/References/BI-20892_proANP_ELISA_References_June_2014.pdf