RESPONSE BIOMEDICAL

INSTRUCTIONS FOR USE

RAMP® NT-proBNP

C1104-1.4

WARNING!

For *in vitro* diagnostic use only

Failure to follow RAMP® test procedures may result in invalid and/or erroneous results. Read the entire Instructions For Use prior to performing test.

INTENDED USE

The RAMP® NT-proBNP Assay is a quantitative immunochromatographic test indicated for use as an *in vitro* diagnostic product to measure N-terminal pro-brain natriuretic peptide (NT-proBNP) levels in EDTA whole blood. The RAMP® NT-proBNP Assay can be used in the clinical laboratory and at the point-of-care where measurement of NT-proBNP aids in the diagnosis and assessment of severity in individuals suspected of having congestive heart failure (CHF) and may aid in the risk stratification of patients with acute coronary syndrome and heart failure. The test may also serve as an aid in the assessment of increased risk of cardiovascular events and mortality in patients at risk for heart failure who have stable coronary artery disease.

WWW.RESPONSEBIO.COM | SPEED. PRECISION. ACCURACY.

IVD

CE

RESPONSE CORPORATE OFFICE

Tel: 1-888-591-5577 (toll free) Tel: 1-604-456-6010 (int'l)

Email: customersupport@responsebio.com

24-HOUR TECHNICAL SUPPORT

Tel: 1-866-525-7267 (toll free) Tel: 1-604-219-6119 (int'l)

Email: techsupport@responsebio.com

Running a test

SUMMARY AND EXPLANATION

Congestive Heart failure (CHF) is a chronic, progressive disease in which the heart muscle weakens and its function becomes impaired, thus impeding the heart's ability to pump enough blood to support the body's metabolic demands. When cardiac muscle is stretched, such as with elevated ventricular filling pressure, the inactive prohormone B-type natriuretic peptide (proBNP) is released and rapidly cleaved into physiologically active BNP and the N-terminal fragment NT-proBNP [1]. Natriuretic peptides can be used for the diagnosis of clinical problems associated with left ventricular dysfunction [2]. The advent of testing for BNPs has improved the ability of physicians to make a qualified diagnosis of heart failure and to monitor the success of treatment [3]. Being able to test the levels of NT-proBNP in patient blood samples is very useful as these levels are indicative of the degree of CHF, and when combined with clinical judgment they provide superior diagnostic performance than clinical judgment alone [4,5]. NT-proBNP has been used for risk stratification of patients with acute coronary syndrome and CHF [6-8]. It has also been shown to aid in the assessment of increased risk of cardiovascular events and mortality in patients at risk for CHF who has stable coronary artery disease [9-11].

Point-of-care (POC) or "near-patient" testing allows for diagnostic assays to be performed at the site of patient care delivery such as the emergency room (ER), chest pain evaluation center, or intensive care unit (ICU). Compared with centralized laboratory testing, POC testing provides for rapid clinical decision making by reducing the time spent ordering tests, collecting and transporting samples, as well as retrieving data.

TEST PRINCIPLE

The RAMP® NT-proBNP test is a quantitative immunochromatographic test for the determination of NT-proBNP in EDTA whole blood. The EDTA whole blood is mixed with buffer and antibody-coated, labeled particles, and applied into the sample well of the test cartridge. The red blood cells are retained in the sample pad and the separated plasma migrates along the strip. Fluorescent-dyed particles coated with anti-NT-proBNP antibodies bind to NT-proBNP, if present in the sample. As the sample migrates along the strip, NT-proBNP bound particles are captured at the detection zone and excess fluorescent-dyed particles are captured at the control zone.

The RAMP® instrument then measures the amount of fluorescence emitted by the complexes bound at the detection zone and at the control zone. Using a ratio between the two fluorescence values, a quantitative reading is calculated. For further information on the use of the instrument, refer to the RAMP® Operator's Manual.

REAGENTS

- The RAMP® test kit contains all the reagents necessary for the quantification of NT-proBNP in EDTA whole blood.
- The sample buffer contains phosphate buffer, animal protein, surfactant, and ProClin® 300 / ProClin® 950 as preservatives.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use.
- For use by qualified personnel per local, state, or Federal regulations or accrediting agency requirements.
- Read the entire instructions for use (IFU) prior to use. Directions should be read and followed carefully, or invalid or erroneous results may occur.
- Do not interchange or mix components of different RAMP® tests, lots or components from other manufacturers.
- Do not use the kit or any kit component beyond the stated expiry date.
- Do not use any visibly damaged components.
- Do not insert a cartridge on which blood or any other fluid is spilled, into the instrument.
- Disposal of all waste materials should be in accordance with local guidelines.
- Exercise standard precautions required for handling all laboratory reagents and patient samples.
- The device contains material of animal origin and should be handled as a potential biohazard.
- The sample buffer provided contains ProClin®, a potential skin sensitizer. Avoid spilling or splashing reagents containing ProClin® on skin or clothing. In case of contact, thoroughly flush with water.

STORAGE AND STABILITY

Store at 2 to 8°C (35 to 46°F). Do not freeze.

Stability

Unopened at 2 to 8°C (35 to 46°F)	Up to the stated expiration date
When stored at 15 to 25°C (59 to 77°F)	14 days

SAMPLE COLLECTION & PREPARATION

- Use ONLY EDTA Whole Blood (Plastic K_2EDTA tubes are recommended). Other sample types and anticoagulants have not been evaluated. Do not use samples that have been frozen.
- Avoid blood samples that show gross hemolysis as these may interfere with the test and cause erroneous results. If this occurs, another blood sample should be obtained and tested.
- Testing must be completed within 2 hours of phlebotomy. However, if this is not possible, the EDTA whole blood can be stored for up to 2 days at 2 to 8°C. If stored, allow blood samples to equilibrate to 18 to 25°C for at least 15 minutes prior to use.

MATERIALS PROVIDED

- 25 pouches, each containing 1 RAMP® test cartridge and 1 test tip
- 25 RAMP® buffer vials
- 1 transfer device for 75 µL
- 1 lot card
- 1 instructions for use (IFU)

MATERIALS REQUIRED (BUT NOT PROVIDED)

- REF: C1100 RAMP® Reader instrument; or
- REF: C2100 RAMP® 200 instrument control module, and
  REF: C3100 RAMP® 200 instrument test module
- REF: C2003 RAMP® Cardiac Controls (optional)
- Optional accessories such as RAMP® printer and/or barcode scanner
- Specimen collection tubes: EDTA (Venous Whole Blood)

Use only the listed RAMP® instruments with this test.

LOT CARD CALIBRATION

Each RAMP® test kit includes a lot card that is individually packaged in an anti-static pouch. The lot card provides information specific to the kit test cartridge lot, including lot number, expiration date, and standard curve information. For further details on loading lot-specific information, see the RAMP® instrument Operator's Manual. No additional calibration beyond insertion of the lot card is necessary. This operation is required only once per test kit lot.

For each new lot, remove the lot card from its pouch and insert it into the lot card slot on the instrument. Once the lot card has been uploaded, return to its pouch and do not discard. Avoid touching the contacts at the end of the lot card.

PROCEDURE

Prior to sample preparation allow all components to come to room temperature for at least 15 minutes.

- Keep the test cartridge and test tip in the sealed foil pouch until ready for use. Once opened, test cartridges and test tips must be used or discarded within 60 minutes.
- The test cartridge, test tip, and buffer vial should be discarded after a single-use. Do not reuse.

1. Prepare RAMP® instrument for test cartridge. Refer to the RAMP® Operator's Manual for detailed instructions on Starting a Test.
2. Ensure that the EDTA whole blood sample is well mixed by gentle inversion.
3. Uncap the buffer vial and place upright on a clean, dry level surface, or in a holder.
4. Open a test pouch and remove the test cartridge and tip. Place the test cartridge on a clean, level surface. Firmly attach the test tip to the supplied transfer device.
5. Before inserting the test tip into the sample, fully depress the transfer device plunger.
6. Insert tip into sample and fully release plunger. The test tip should fill with 75 µL of blood.
7. Immediately transfer the filled test tip into the buffer vial close to, but not touching, the bottom.
8. Mix sample slowly by fully pressing and releasing the plunger 10 times; while keeping the tip submerged in the buffer for optimal mixing and to minimize air bubbles.
9. Once mixing is complete, draw 75 µL of sample into the test tip by releasing the plunger one final time and immediately dispense liquid into the sample well of the test cartridge. Small droplets may remain in the tip; this is expected.
10. Immediately insert the test cartridge fully into the instrument and press until firm resistance is felt.
11. The instrument will draw the cartridge in and test development will begin.
12. The instrument will analyze the cartridge and report the result in approximately 15 minutes.
13. Record the result, if required. For additional information on printing and/or uploading results, please refer to the Operator's Manual.
14. Remove the used test cartridge and discard all used test components according to local biohazard procedures. DO NOT reuse.

For additional information on the general operation and troubleshooting of the instrument, please refer to the RAMP® Operator's Manual.

QUALITY CONTROL

Refer to the RAMP® Operator's Manual for full details on quality control operation and troubleshooting.

SYSTEM QUALITY CONTROL

The RAMP® instrument has error checking and self-diagnostic functions (Internal Quality Control (IQC)) that assure system integrity. These include algorithms and measurements used to confirm acceptable operator technique, sample handling, and test performance. Frequency of IQC may be programmed at desired intervals.

Valid results are displayed only after all performance requirements have been met.

PROCEDURAL CONTROLS

- Each RAMP® test has built-in controls. Test cartridges have a control zone that is scanned as part of the test protocol to ensure proper sample flow.
- Control limits for each lot of test cartridges are established during the manufacturing process and are incorporated in the test-specific lot parameters. If a control result does not meet specifications, the sample result is not reported and a message is displayed.

LIQUID QUALITY CONTROL (LQC)

- It is recommended that quality control materials be run with the RAMP® test in conformance with Federal, state and local requirements for quality control testing.
- While the running of commercial control materials are recommended, it is not a requirement to use, or assure, performance of the RAMP® test unless specified by local regulations or institutional requirements.
- To run a LQC sample, follow the instructions under the "Procedure" section in this IFU. Treat the control as a whole blood sample.

TEST RUN MESSAGES

When the RAMP® instrument is unable to continue a specific task it will emit an audio alarm and display a message. Refer to the RAMP® Operator's Manual 'Troubleshooting Guide' section for a full description of all messages. If repeated tests give unexpected results, contact Response Biomedical Technical Support for assistance.

LIMITATIONS

- For diagnostic purposes, the patient's medical history, clinical examination and other findings should always be assessed in conjunction with the RAMP® test results. A test result that is inconsistent with the clinical signs and symptoms should be interpreted with caution.
- Factors such as technical or procedural errors or the presence of substances in blood specimens other than those that have been evaluated (see Interference section of this IFU), may interfere with the RAMP® test and cause erroneous results.
- As with any immunoassay, patient specimens may contain heterophilic antibodies that may result in either falsely elevated or depressed results. Presence of these antibodies may be due to elevated levels of rheumatoid factor, treatment with mouse monoclonal antibodies for diagnostic or therapeutic purposes, or other undetermined factors. The RAMP® test has been formulated to reduce the effects of heterophilic antibodies, but complete elimination of heterophilic interference from all samples cannot be guaranteed.

TEST CUT-OFF AND EXPECTED VALUES

The test cut-offs validated for the RAMP® NT-proBNP test are 125 ng/L for < 75 years of age and 450 ng/L for ≥ 75 years of age. Each laboratory should investigate the transferability of the expected values to its own patient population and, if necessary, determine its own reference ranges

PERFORMANCE CHARACTERISTICS

MEASUREMENT RANGE

18 to 35,000 ng/L

NT-proBNP levels in excess of 35,000 ng/L are reported as greater than > 35,000 ng/L, values less than 18 ng/L should be reported as < 18 ng/L.

HOOK EFFECT

No high dose hook effect was observed for the RAMP® NT-proBNP test up to the highest level tested (350,000 ng/L NT-proBNP).

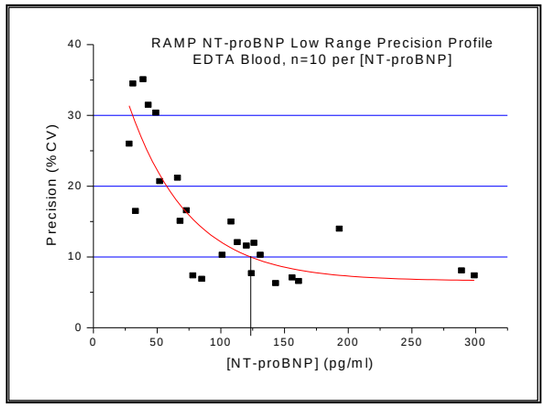
DETECTION LIMIT

The lower limit of detection (LLD) is defined as the analyte concentration corresponding to the mean (n=20) plus 2 standard deviations of the zero. The LLD is 18 ng/L NT-proBNP.

Another characteristic of analytical measurement is the functional sensitivity, which is defined as the NT-proBNP level at which the test method displays a particular coefficient of

variation (%CV). The 10% functional sensitivity for the RAMP® NT-proBNP test was determined from whole blood analysis to be 123 ng/L NT-proBNP.

NT-proBNP Low Range Precision Profile, EDTA Whole Blood



PRECISION

The within-run and total precision of the RAMP® NT-proBNP test were determined by one operator assaying duplicates of 3 concentrations of control material twice each day over a 10-day period. The mean, standard deviation and % CV were calculated for each reported concentration of NT-proBNP. The results of this precision analysis are shown below:

	NT-proBNP Standards Mean Concentration [ng/L]		
	140	449	1675
Within-run [%]	9.4	6.4	5.5
Total [%]	10.3	9.8	8.9

Duplicate Precision Comparison at Clinical Sites

Subjects enrolled in the precision study were a subset of the subjects enrolled to the method comparison study. 439 subjects were enrolled into the precision study. The samples were selected from those with concentrations ranging between 5 and 35,000 ng/L of NT-proBNP. Of these samples, 219 were diagnosed with CHF based on individual hospital criteria (135 males and 84 females) and 220 were reference group subjects (90 males and 130 females). The correlation data for replicate result 2 vs result 1 for the RAMP® NT-proBNP test is presented in the table below.

Method	Slope	Intercept [ng/L]	Correlation Coefficient [r]
RAMP®	1.002	-3.11	0.99
95% CI	0.996 to 1.012	-5.80 to 0.67	0.99 to 1.00

LINEARITY

A high endogenous clinical EDTA plasma sample (23,428 ng/L) was serially diluted five times (n=5) using NT-proBNP depleted matrix. The linearity and percent recovery were determined by assaying three (3) replicates of each concentration and baseline. Linear regression analysis of actual NT-proBNP concentration versus expected NT-proBNP concentration resulted in an R-value of 1.00, a slope of 0.999 and an offset of -68 ng/L. The recovery of NT-proBNP antigen at the five dilutions was 631, 1362, 3000, 5848 and 11,438 ng/L, and ranged from 86 to 102% with an average of 96%.

A mid-level endogenous clinical EDTA plasma sample (12,236 ng/L) was serially diluted five times (n=5) using NT-proBNP depleted matrix. The linearity and percent recovery were determined by assaying three (3) replicates of each concentration and baseline. Linear regression analysis of actual NT-proBNP concentration versus expected NT-proBNP concentration resulted in an R-value of 1.00, a slope of 1.007 and an offset of -86 ng/L. The recovery of NT-proBNP antigen at the five (5) dilutions was 363, 671, 1409, 2959 and 6111 ng/L, and ranged from 88 to 100% with an average of 94%.

INTERFERENCE

Hemoglobin, triglycerides, bilirubin, cholesterol, and heparin at levels representing high physiological concentrations were tested for possible interference. No interference was observed when tested at the concentrations up to, and including those shown in the following table:

Compound	Concentration
Hemoglobin	2 g/dL
Triglycerides	4 g/dL
Bilirubin	35 mg/dL
Cholesterol	500 mg/dL
Heparin	104 IU/mL

ANALYTICAL SPECIFICITY

Human anti-mouse antibodies (HAMA) and Rheumatoid Factor (RhF) appear to have minimal cross-reactivity with RAMP® NT-proBNP. Possible cross-reactivity of other substances was evaluated by spiking different concentrations of the potential cross-reactants into EDTA blood, which had NT-proBNP added. No cross-reactivity was observed with the RAMP® NT-proBNP test up to the maximum levels tested for the compounds listed in the following table:

Compound	Concentration
ANP_{28}	3.1µg/mL
BNP_{32}	3.5 µg/mL
CNP_{22}	2.2 µg/mL
$preproANP_{26-55}$	3.5 µg/mL
$preproANP_{56-92}$	1 ng/mL
$preproANP_{104-123}$	1 ng/mL
Aldosterone	0.6 ng/mL
Angiotensin I	0.6 ng/mL
Angiotensin II	0.6 ng/mL
Angiotensin III	1 ng/mL
Endothelin	20 ng/L
Arg-Vasopressin	1 ng/mL
Renin	50 ng/mL
Andrenomedullin	1 ng/mL
Urodilatin	3.5 µg/mL

CLINICAL EVALUATIONS

METHOD COMPARISON

Six hundred and six (606) subjects were enrolled in the method comparison clinical trial. EDTA and heparin whole blood samples were obtained for each of these subjects. An aliquot of EDTA whole blood was used for the RAMP® NT-proBNP test and heparinized plasma was prepared for the Roche Elecsys proBNP assay. From these analyses it was determined that 540 samples contained between 5 and 35,000 ng/L of NT-proBNP. Of these, 256 were diagnosed with CHF based on individual hospital criteria (160 males and 96 females) and 284 were reference group subjects (124 males and 160 females). The correlation data of RAMP® NT-proBNP vs. Elecsys proBNP is presented in the table below.

Comparative Method	Slope	Intercept [ng/L]	Correlation Coefficient [r]
Elecsys proBNP	1.005	14.83	0.98
95% CI[a]	0.973 to 1.042	7.35 to 19.83	0.98 to 0.98

a) Confidence interval

CLINICAL SENSITIVITY & SPECIFICITY

Each of the enrolled study subjects were diagnosed as either CHF or non-CHF (reference) based on individual hospital criteria. Clinical concordance was calculated comparing the RAMP® NT-proBNP test results to the Roche Elecsys proBNP assay results for both the reference and CHF groups using age specific cut offs of 125 ng/L for subjects < 75 years of age and 450 ng/L for subjects ≥ 75 years of age. The data is presented in the table below.

Age Stratified Sensitivity: 125/450 ng/L for Ages <75 and >75

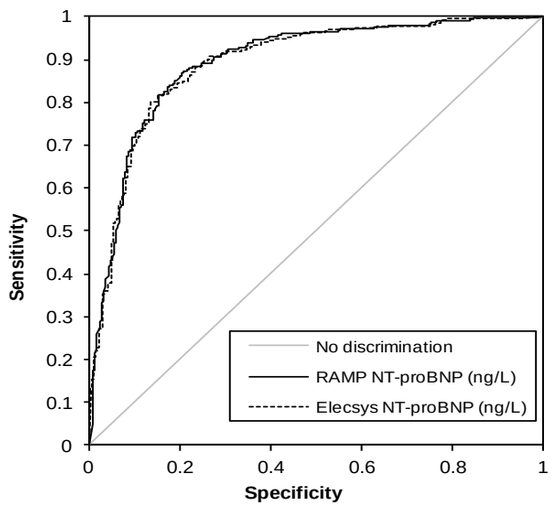
Male		
Age (years)	< 75	≥ 75
n	267	63
Sensitivity	82.3	74.2
95% CI	(74.2 to 90.0)	(62.7 to 86.3)
Female		
Age (years)	< 75	≥ 75
n	215	61
Sensitivity	94.8	91.1
95% CI	(89.7 to 96.7)	(80.8 to 95.9)

Sensitivity & Specificity by Cohort

	Non-CHF		CHF	
	%	95% CI	%	95% CI
Sensitivity	95	88.6 to 97.5	100	98.5 to 100
Specificity	91	86.2 to 93.8	88	69.0 to 95.7
PV+	83	75.7 to 88.7	99	96.5 to 99.6
PV-	97	93.9 to 98.7	100	84.5 to 100
Concordance	92	88.5 to 94.4	99	96.8 to 99.6

RECEIVER OPERATOR CHARACTERISTIC (ROC)

The ROC analyses for both the RAMP® NT-proBNP and Roche Elecsys proBNP tests for the clinical trial population are shown below. The area under the curve (AUC) for both the RAMP® NT-proBNP test and Elecsys proBNP test is 0.876.



CHF POPULATION BY NYHA CLASSIFICATION

The 271 subjects diagnosed with heart failure were evaluated using the RAMP® NT-proBNP test. The descriptive statistics for NT-proBNP concentrations are presented according to NYHA Functional Classification in the table below.

NYHA Class	I	II	III	IV
All				
n	35	87	83	66
Mean	1149	2886	6101	9323
SD	1512	4921	7013	9366
Median	514	1325	3618	6628
95th Percentile	5552	8201	20668	30696
Male				
n	22	55	54	40
Mean	1169	3002	6119	10063
SD	1528	5222	6978	10048
Median	583	1310	3627	5772
95th Percentile	4002	18949	22745	31539
Female				
n	13	32	29	26
Mean	1115	2685	6066	8186
SD	1546	4428	7201	8267
Median	493	1478	3598	6937
95th Percentile	5552	8201	17393	27702

NON-CHF AND CHF GROUP DESCRIPTIVE STATISTICS

The overall incidence of disease in the presenting population (n=606) included 43% (262) subjects with hypertension, 34% (208) who presented with shortness of breath, 22% (136) with diabetes, 15% (91) with pulmonary disorders, 12% (74) with coronary disease, 9% (55) with atrial fibrillation, 5% (29) with renal failure, 21% (127) were healthy, and the remainder had diagnoses not believed to be cardiac related (hepatitis, HIV, cancer, etc.).

The circulating NT-proBNP concentration was determined in 606 individuals with and without CHF. The CHF subjects included those with prior established heart failure that were not acutely destabilized at the time of enrollment (and thus similar to those who might be tested in the outpatient setting). The descriptive statistics for the Non-CHF (with and without co-morbidities) and the CHF groups are presented in the following tables:

Non-CHF Subjects – RAMP® Results [ng/L]

	No Co-morbidity		With Co-morbidity	
Age (years)	< 75	≥ 75	< 75	≥ 75
n	123	4	158	50
Mean	136.7	91.5	704	869
SD	624	34	3026	1284
Median	57	84	126	403
95th Percentile	200	138	3068	3115
% < 125 ng/L	82	-	50	-
% < 450 ng/L	-	100	-	52

CHF Subjects – RAMP® Results [ng/L]

	With Co-morbidity	
Age (years)	< 75	≥ 75
n	195	76
Mean	5014	5725
SD	7599	6212
Median	2108	3418
95th Percentile	27702	20668
% < 125 ng/L	10	-
% < 450 ng/L	-	1

REFERENCES

[1]. Hall C. Essential biochemistry and physiology of (NT-pro)BNP. Eur J Heart Fail. 2004;6:257-260.

[2]. Richards AM, Nicholls GM, Yandle TG, et al. Plasma N-terminal pro-brain natriuretic peptide and adrenomedullin: New hormonal predictors of left ventricular function and prognosis after myocardial infarction. Circ. 1998;97:1921-1929.

[3]. Rademaker MT, and Richards MA. Cardiac natriuretic peptides for cardiac health. Clin Sci. 2005;108:23-36.

[4]. Januzzi JL, Camargo CA, Anwaruddin S, et al. The N-terminal Pro-BNP investigation of dyspnea in the emergency department (PRIDE) study. Am J Cardiol. 2005;95:948-954.

[5]. Baggish AL, Cameron R, Anwaruddin S, et al. A clinical and biochemical critical pathway for the evaluation of patients with suspected acute congestive heart failure. The proBNP investigation of dyspnea in the emergency department (PRIDE) algorithm. Crit Path Cardiol. 2004;3:171-176.

[6]. James SK, Lindahl B, Siegbahn A, et al. N-terminal pro-brain natriuretic peptide and other risk markers for the separate prediction of mortality and subsequent myocardial infarction in patients with unstable coronary artery disease: a global utilization of strategies to open occluded arteries (GUSTO)-IV substudy. Circ. 2003;108:275-81.

[7]. Jernberg T, Stridsberg M, Venge P, et al. N-terminal pro brain natriuretic peptide on admission for early risk stratification of patients with chest pain and no ST-segment elevation. J Am Coll Cardiol. 2002;40:437-45.

[8]. Fisher C, Berry C, Blue L, et al. N-terminal pro B type natriuretic peptide, but not the new putative cardiac hormone relaxin, predicts prognosis in patients with chronic heart failure. Heart. 2003;89:879-81.

[9]. Schnabel R, Rupprecht HJ, Lackner KJ, et al. Analysis of N-terminal-pro-brain natriuretic peptide and C-reactive protein for risk stratification in stable and unstable coronary artery disease: Results from the AtheroGene study. Eur Heart J. 2005;26:241-249.

[10.] Kragelund C, Groenning B, Kober L, et al. N-terminal pro-B-type natriuretic peptide and long-term mortality in stable coronary heart disease. N Eng J Med. 2005;352:666-675.

[11.] Ndrepepa G, Braun S, Niemoller K, et al. Prognostic value of N-terminal pro-brain natriuretic peptide in patients with chronic stable angina. Circ. 2005;112:2102-2107.

GLOSSARY OF SYMBOLS

EC REP Authorized Representative in European Community	LOT Batch Code	REF Catalogue Number
Caution	CE Mark	Consult Instructions for Use
Contains Sufficient for <n>Tests	Do Not Reuse	IVD In vitro Diagnostic Medical Device
Harmful, Irritant	Manufacturer	Temperature Limit
Use-by Date		

PRODUCT SUPPORT / ASSISTANCE

If you have any questions regarding the use of this product please contact Response Biomedical Corp. Technical Support:

- Within US or Canada (+1.866.525.7267)
- Outside US or Canada (+1.604.219.6119)
- By email at techsupport@responsebio.com

MANUFACTURER

RESPONSE BIOMEDICAL

Response Biomedical Corp.
1781 – 75th Avenue W
Vancouver, BC, Canada V6P 6P2
T. +1.604.456.6010
T. +1.888.591.5577 (Toll-free North America)
E. customersupport@responsebio.com

2014-03, V 1.4, English

REF C1104