RESPONSE BIOMEDICAL

INSTRUCTIONS FOR USE

C1102-1.4

RAMP® CK-MB

WARNING!

For *in vitro* diagnotic 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® CK-MB Assay is a quantitative immunochromatographic test indicated for use as an *in vitro* diagnostic product used to measure CK-MB levels in EDTA whole blood. Measurement of CK-MB aids in the rapid diagnosis of acute myocardial infarction (AMI). The RAMP® CK-MB Assay is not intended to monitor reperfusion patients. The RAMP® CK-MB Assay is intended to be used only to prioritize patient management for those suspected of AMI.

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RESPONSE CORPORATE OFFICE

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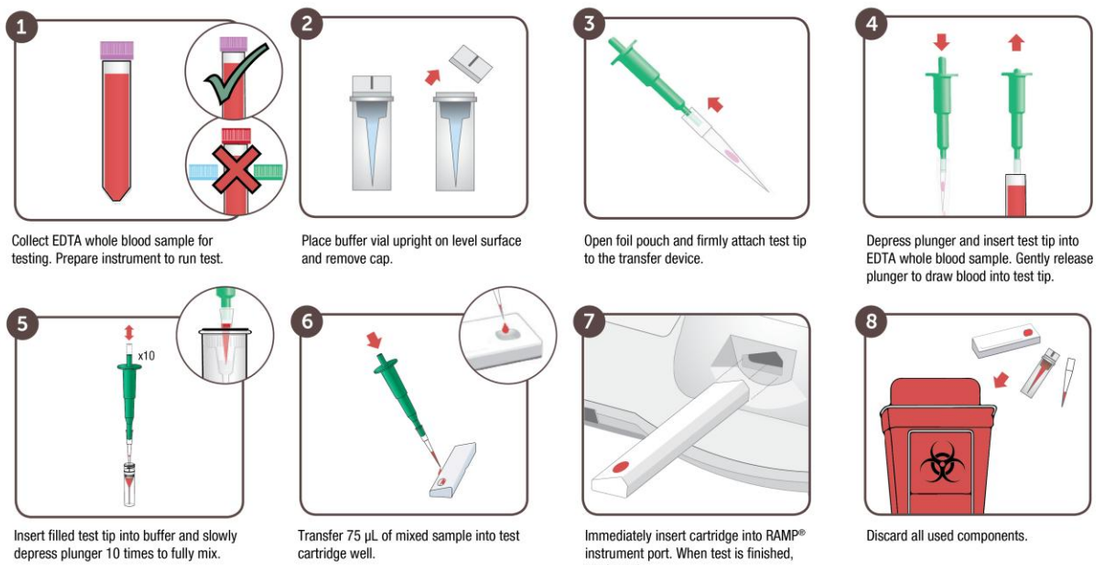
Running a test



SUMMARY AND EXPLANATION

Upon Acute Myocardial Infarction (AMI), there are a number of biochemical markers that are released into the bloodstream. One of these markers is the MB isoform of creatine kinase (CK-MB). CK-MB is found primarily in cardiac tissue with substantially lower levels in skeletal muscle [1 to 5]. CK-MB levels become elevated within 6 hours after onset of chest pain returning to normal levels within 48 to 72 hours [4].

TEST PRINCIPLE

The RAMP® CK-MB test is a quantitative immunochromatographic test for the determination of CK-MB 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-CK-MB antibodies bind to CK-MB, if present in the sample. As the sample migrates along the strip, CK-MB-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 CK-MB 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 US customers, the RAMP® CK-MB test must be operated in a laboratory setting when used with the RAMP® 200.
- 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, RAMP® 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 15to 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.
- 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 12 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; the results of the RAMP® CK-MB test are not to be used to classify the extent of myocardial necrosis or to monitor reperfusion patients.
- 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.
- When RAMP® results are elevated; the physician may choose to employ a ratio CK-MB / total CK determination by the hospital laboratory to aid in the differential diagnosis of AMI.
- Caution: Federal law restricts this device to sale by or on the order of a licensed healthcare practitioner (U.S. only).

TEST CUT-OFF AND EXPECTED VALUES

According to the World Health Organization (WHO) definition, the diagnosis of acute myocardial infarction (AMI) is based on the presence of at least two of the following three criteria: a clinical history of ischemic-type chest discomfort; changes on serially obtained electrocardiographic tracings; and a rise and fall in serum cardiac markers [6].

Historically, the amount of total CK has been used as an aid in the diagnosis of AMI. Although CK-MB (measured by mass assay) is less tissue-specific than cardiac troponin, the data documenting its clinical specificity for irreversible injury are more robust. Measurement of total CK is not recommended for the routine diagnosis of AMI, because of the wide tissue distribution of this enzyme. Nevertheless, total CK has a long history and if used should be combined with a more sensitive biomarker, such as cardiac troponin or CK-MB [7]. Serial determination of CK-MB has long been considered the "gold standard" for AMI diagnosis [5,8].

One hundred and eighty (180) normal individuals were enrolled in the expected values clinical trial. RAMP® CK-MB expected normal values ranged from 0.00 to 3.74 ng/mL CK-MB. The percentile ranking is presented in the table below:

Percentile	ng/mL
5th (LLN)	0.00
50th	0.78
90th	2.87
95th (ULN)	3.74
97.5th	4.99

Each laboratory should investigate the transferability of the expected values to its own patient population and, if necessary, determine its own reference ranges. The RAMP® CK-MB test is not intended to monitor reperfusion patients.

The RAMP® CK-MB test is intended to be used only to prioritize patient management for those suspected of AMI.

PERFORMANCE CHARACTERISTICS

MEASUREMENT RANGE

0.32 to 80 ng/mL

CK-MB levels in excess of 80 ng/mL are reported as greater than > 80 ng/mL, values less than 0.32 ng/mL should be reported as < 0.32 ng/mL.

HOOK EFFECT

No high dose hook effect was observed for the RAMP® CK-MB test up to the highest level tested (1,000 ng/mL CK-MB).

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 0.32 ng/mL CK-MB.

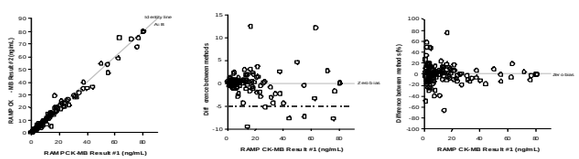
PRECISION

The within-run and total precision of the RAMP® CK-MB test were determined by one operator assaying duplicates of three (3) standards twice each day over ten (10) days. The mean, standard deviation and % CV were calculated for each reported concentration of CK-MB.

	CK-MB Standards Mean Concentration [ng/mL]		
	7.19	14.29	25.06
Within-run [%]	7.7	7.8	4.8
Total [%]	8.6	8.5	6.9

CLINICAL SITE EVALUATIONS OF ANALYTICAL PERFORMANCE:

STANDARD ERROR OF THE ESTIMATE BETWEEN RUNS



n	128	
bias	-0.145	
95 % CI	-0.595 to 0.305	
95 % limits of agreement		**95 % CI**
lower	-5.190	-5.952 to -4.428
upper	4.900	4.138 to 5.662

Subjects enrolled in the precision study were a subset of the subjects enrolled to the method comparison study. 184 total subjects were enrolled; of these, 55 were normal individuals (28 males and 27 females) and 129 were patients suspected of AMI based on the individual hospital criteria (76 males and 53 females). The samples were stored refrigerated for up to one day between analyses. The data were reviewed and one outlier removed from the combined and suspect AMI populations. Correlation (linear regression) for replicate result 2 vs result 1 for the RAMP® CK-MB test is presented below. The standard error of the estimate is Sy.y = 2.57.

Population	n	Sy.x	Slope	Intercept [ng/mL]	Correlation Coefficient [r]
Combined	183	2.16	0.989	0.021	0.993
Suspect AMI	128	2.57	0.989	0.051	0.993
Normal	55	0.38	0.926	0.056	0.959

LINEARITY

CK-MB antigen concentrations of 2.5, 5.0, 10.0, 20.0, 40.0 and 60.0 ng/mL were prepared in normal donor EDTA whole blood. The linearity and percent recovery were determined by assaying five replicates of each concentration and baseline. The mean, standard deviation and %CV of replicates were calculated for each concentration. Linear regression analysis of actual CK-MB concentration versus expected CK-MB concentration resulted with an R = 0.999 and a slope of 1.05 with an offset of 0.098. The recovery of spiked CK-MB antigen at the six concentrations ranged from 99 to 111% with an average of 106%.

INTERFERENCE

Potentially interfering substances were evaluated by spiking different concentrations of interferents in normal donor EDTA whole blood with CK-MB added. Different blood samples were used for each interferent. Interference was evaluated by calculating the CK-MB concentration of interferent-spiked blood, expressed as a percentage of the CK-MB concentration of the un-spiked (no interferent) blood sample. Hemoglobin, triglycerides, bilirubin, cholesterol, and heparin at levels presenting 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	2000 mg/dL
Triglyceride	3000 mg/dL
Bilirubin	80 mg/dL
Cholesterol	500 mg/dL
Heparin	104 IU/mL

ANALYTICAL SPECIFICITY

Potentially cross-reactive substances were evaluated by spiking different concentrations of each potential cross-reactant into a CK-MB-free matrix. CK-MM up to 50,000 ng/mL and CK-BB up to 1000 ng/mL appear to have no cross-reactivity with the RAMP® CK-MB test. Human anti-mouse antibodies (HAMA), human anti-goat antibodies (HAGA), human anti-rabbit antibodies (HARA) and Rheumatoid Factor (RhF) appear to have limited cross-reactivity with the RAMP® CK-MB test.

CLINICAL EVALUATIONS

METHOD COMPARISON

365 subjects were enrolled in the method comparison clinical trial. Of these subjects, 180 were normal individuals (84 males and 96 females) and 185 were suspected of AMI based on the individual hospital criteria (115 males and 70 females). EDTA and heparin whole blood samples were obtained for each of these subjects. All normal subjects were consented. Waste samples were used for the subjects suspected of AMI. An aliquot of the EDTA whole blood sample was taken for the RAMP® CK-MB test and heparinized plasma was prepared for the Dade Behring Dimension CK-MB assay. The data was winsorized to account for the differing reportable ranges and two outliers were removed from the suspect AMI samples. The correlation data is presented in the table below.

Population	n	Sy.x	Slope	Intercept [ng/mL]	Correlation Coefficient [r]
Combined	363	3.11	0.966	0.600	0.986
Suspect AMI	183	4.28	0.955	1.207	0.984

CLINICAL SENSITIVITY & SPECIFICITY

The sensitivity, specificity, and percent agreement of all samples were calculated comparing a clinical cutoff of 6.0 ng/mL CK-MB for the RAMP® CK-MB test to the published clinical cutoff of 5.0 ng/mL CK-MB presented in the Dade Dimension package insert. The RAMP® CK-MB test demonstrates good sensitivity, specificity, and percent agreement when compared with this reference method. The data is presented in the table below.

	n	[%]	s.e.[a]	95% CI[b]	
Sensitivity	101	96.04	1.94	92.24	99.84
Specificity	264	97.73	0.92	95.93	99.53
PV[c] +	103	94.18	2.31	89.65	98.70
PV -	262	98.47	0.76	96.99	99.96
Concordance	365	97.26	0.85	95.59	98.94

a) s.e = Standard error
b) CI = Confidence interval
C) PV = Predictive value

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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	Rx Only Prescription Use Only (U.S. Only)
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

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