INSTRUCTIONS FOR USE
ONE STEP TEST
Myoglobin/CK-MB/Troponin I Cardiac Markers
Detection in Whole Blood / Serum / Plasma

TOYO DIAGNOSTICS

Only for professional *in vitro* diagnostic use

Product Code : TMT01
Myoglobin/CK-MB/Troponin I Combo Test Device detects cardiac markers Myoglobin, CK-MB and Troponin I in human whole blood, serum and plasma.

BACKROUND INFORMATION

Myoglobin (MYO), Creatine Kinase MB (CK-MB) and cardiac Troponin I (cTnI) are proteins released into the bloodstream after cardiac injury. Myoglobin is a hemeprotein normally found in skeletal and cardiac muscle with a molecular weight of 17.8 kDa. It constitutes about 2 percent of total muscle protein and is responsible for transporting oxygen within muscle cells. When muscle cells are damaged, Myoglobin is released into the blood rapidly due to its relatively small size. The level of Myoglobin increases measurably above baseline within 2-4 hours post-infarct, peaking at 9-12 hours, and returning to baseline within 24-36 hours. CK-MB is an enzyme also present in the cardiac muscle, with a molecular weight of 87.0 kDa. Creatine Kinase is a dimeric molecule formed from two subunits designated as “M” and “B”, which combine to form three different isoenzymes, CK-MM, CK-BB and CK-MB. CK-MB is the isoenzyme of Creatine Kinase most involved in the metabolism of cardiac muscle tissue. The release of CK-MB into the blood following an MI can be detected within 3-8 hours after the onset of symptoms. It peaks within 9 to 30 hours, and returns to baseline levels within 48 to 72 hours. Cardiac Troponin I is a protein found in cardiac muscle, with a molecular weight of 22.5 kDa. Troponin I is part of a three subunit complex comprised of Troponin T and Troponin C. Along with tropomyosin, this structural complex forms the main component that regulates the calcium sensitive ATPase activity of actomyosin in striated skeletal and cardiac muscle. After cardiac injury occurs, Troponin I is released into the blood 4-6 hours after the onset of pain. The release pattern of Troponin I is similar to CK-MB, but while CK-MB levels return to normal after 72 hours, Troponin I remains elevated for 6-10 days, thus providing for a longer window of detection for cardiac injury.

INTENDED USE

Myoglobin/CK-MB/Troponin I Combo Test Device is a rapid immunochromatographic assay for qualitative detection of human cardiac marker Myoglobin, CK-MB and Troponin I in human whole blood / serum / plasma to aid diagnosis of myocardial infarction (MI).

REAGENTS

The test contains anti-Myoglobin antibody coated particles, anti-CK-MB antibody coated particles, anti-Troponin I antibody coated particles, and capture antibodies immobilized on the membrane.

METHOD

Myoglobin/CK-MB/Troponin I Combo Test Device is a rapid, qualitative, immunochromatographic assay for the detection of Myoglobin, CK-MB and Troponin I in human whole blood / serum / plasma samples. Specific capture antibodies are immobilized on each test area of the test. While performing the test; whole blood / serum / plasma sample dropped to the sample well and reacts with the specific antibody coated particles. This complex migrates to the other end of the membrane by capillary action. If Myoglobin and/or CK-MB and/or Troponin I present in the sample, it binds to specific capture antibodies in the separate and specific test areas and create a visible, colored signal that means the test result is positive. If the sample does not contain Myoglobin and/or CK-MB and/or Troponin I, specific colored line does not appear in the test area. This means the test result is negative. As a procedural control, a colored line always appears in the “C” control area indicating that proper volume of sample has been introduced and membrane wicking has occurred.

PRECAUTIONS AND LIMITATIONS

1.For professional and *in vitro* diagnostic use only.
2.Do not use test kit beyond expiry date. The test device is single use. Do not reuse.
3.The test device should remain in its original sealed pouch until usage. Do not use the test if the seal is broken or the pouch is damaged.
4.Wear disposable gloves while performing the test.
5.Use a new dropper for each sample.
6.All patient samples should be handled as taking capable of transmitting disease into consideration. Observe established precautions against microbiological hazards throughout all procedures and follow the standard procedures for proper disposal of samples.
7.Unusually high titers of heterophile antibodies or rheumatoid factor (RF) may affect the results. Even if test results are positive, further clinical evaluation should beconsidered with other clinical information available to the physician.
8.This test will indicate only the presence or absence of Myoglobin, CK-MB and Troponin I in the sample, and should not be used as the only basis for the diagnosis of myocardial infarction.
As with all diagnostic tests, it should be kept in mind that an identification diagnosis can't be based on a single test result. Diagnosis can only be reached by an expert after the evaluation of all clinical and laboratory findings.
9.If the test result is negative and clinical symptoms persist, additional testing using other clinical methods is recommended. A negative result does not at any time preclude the possibility of myocardial infarction.

STORAGE

Test device should be kept away from direct sunlight, moisture, heat and radiation sources.
Store at 4 - 30°C (39 - 86°F). Do not freeze.
The test in the original packaging retains stable until expiry date at storage conditions. The test device should be used in maximum one hour after the foil is opened.

SAMPLE COLLECTION AND PREPARATION

The test can be performed using whole blood, serum or plasma. To avoid hemolysis, serum or plasma should be separated from blood as soon as possible.

For Whole Blood Samples: Test should be performed immediately with whole blood samples. Otherwise, whole blood samples should be stored at 2 - 8 °C with anticoagulants (EDTA, heparin, citrate should be used) to avoid coagulation until they are being tested in a period of 2 days after collection.

For Serum Samples: Collect blood into a collection tube without anticoagulant, leave to settle for 30 minutes for blood coagulation and then centrifuge the blood. At the end of centrifuge period remaining supernatant is used as serum.

For Plasma Samples: Collect blood into a collection tube with anticoagulants (EDTA, heparin, citrate should be used) to avoid coagulation of blood sample and then centrifuge the blood. At the end of centrifuge period supernatant is used as plasma.

Do not use turbid, hemolyzed samples. If the sample cannot be tested on the day of collection, store the serum, plasma samples in a refrigerator or freezer. Do not freeze and thaw the serum, plasma samples repeatedly. Do not freeze whole blood sample. Bring the samples to room temperature before testing. Frozen samples must be completely thawed and mixed well prior to testing. Turbid test samples should be centrifuged. Using of frozen and thawed samples should be avoided whenever possible, due to the blocking of the membrane by the debris.

Kit components : Test devices, droppers, diluents (for whole blood samples only) and instructions for use.
Additional materials required but not provided : Sample collection tube, centrifuge and timer, lancet (for only fingerstick whole blood), heparinized dispensing bulbs and capillary tubes (for only fingerstick whole blood).
Additional materials recommended but not provided : Micropipettes to deliver mentioned amount of sample in the test procedure, negative and positive control materials.

TEST PROCEDURE

1. Take the test device out of its pouch. Bring the tests and whole blood / serum /plasma samples to room temperature.
2. **For Whole Blood Samples:** Draw whole blood into dropper and put 3 drops (90 µl) into the sample well of the cassette. Immediately after, 1 drop (~40µL) of diluent are added into the sample well and allowed to soak in.
For Serum / Plasma Samples: Draw serum / plasma into dropper and put 2 drops (60 µl) into the sample well of the cassette. Do not use diluent for serum / plasma samples. Avoid the formation of any air bubbles.
3. Depending on the Myoglobin, CK-MB and Troponin I concentrations in the sample, the test can react even in 5 minutes. Results should be read at 10 minutes as shown below. Results forming after 20 minutes should be regarded as invalid

INTERPRETATION OF RESULTS

Negative : Only one colored line is visible in “C” area, indicating that concentrations of Myoglobin, CK-MB and Troponin I are below the minimum detection levels.
Positive : One colored line is visible in control area “C” and one or more colored lines are visible in test areas, indicating that concentration of cardiac markers are above the minimum detection levels.
Low concentration of cardiac markers may cause a faint line in test area. Even such a faint line in test areas should be regarded as “positive”.
Invalid : No colored line is visible or only one, two or three colored lines are visible in test areas; test should be repeated using a new test device.

QUALITY CONTROL

Tests have built in procedural quality control features. When the test is complete, the user will see a colored line in the “C” area of the test on negative samples and a colored line or lines in the test and control area “C” on positive samples. The appearance of the control “C” line is considered as an internal procedural control. This line indicates that sufficient volume of sample was added as well as valid test result. It is recommended that a negative control and a positive control be used to verify proper test performance as an external control. Users should follow appropriate federal, state and local guidelines concerning the external quality controls.

PERFORMANCE EVALUATION

Sensitivity and Specificity
The Myoglobin/CK-MB/Troponin I Combo Test Device has been evaluated with a leading commercial Myoglobin/CKMB/Troponin I EIA test using clinical specimens.

Myoglobin /CK-MB / Troponin I Combo Test Device - EIA

Method		EIA		Total Results
Myoglobin Test	Results	Positive	Negative	
	Positive	70	8	78
	Negative	0	352	352
Total Results		70	360	430

Sensitivity: 100%
Specificity: 98%
+ Predictive V: 90 %
- Predictive V: 100%

Myoglobin /CK-MB / Troponin I Combo Test Device - EIA

Method		EIA		Total Results
CK-MB Test	Results	Positive	Negative	
	Positive	68	1	61
	Negative	0	380	380
Total Results		68	381	441

Sensitivity: 100%
Specificity: 99,7%
+ Predictive V: 98,4 %
- Predictive V: 100%

Myoglobin /CK-MB / Troponin I Combo Test Device - EIA

Method		EIA		Total Results
Troponin I Test	Results	Positive	Negative	
	Positive	318	6	324
	Negative	2	431	433
Total Results		320	437	757

Sensitivity: 99,4%
Specificity: 98,2%
+ Predictive V: 98,6 %
- Predictive V: 99,5%

Cut off
Myoglobin: 50 ng/mL **CK-MB:** 5 ng/mL **Troponin I:** 0,5 ng/mL

Intra-Assay
Within-run precision has been determined by using replicates of 15 tests using Myoglobin specimen levels at 0 ng/mL, 50 ng/mL, 100 ng/mL, 200 ng/mL and 400 ng/mL, CK-MB specimen levels at 0 ng/mL, 5 ng/mL, 10 ng/mL, 20 ng/mL and 40 ng/mL and Troponin I specimen levels at 0 ng/mL, 2 ng/mL, 5 ng/mL, 10 ng/mL and 20 ng/mL. The specimens were correctly identified >99% of the time.

Inter-Assay
Between-run precision has been determined by 3 independent assays on the same fifteen specimens: 0 ng/mL, 50 ng/mL, 100 ng/mL, 200 ng/mL and 400 ng/mL of Myoglobin, 0 ng/mL, 5 ng/mL, 10 ng/mL, 20 ng/mL, and 40 ng/mL of CK-MB and 0 ng/mL, 2 ng/mL, 5 ng/mL, 10 ng/mL and 20 ng/mL of Troponin I. Myoglobin/CK-MB/ Troponin I Combo Test Device have been tested using these specimens. The specimens were correctly identified >99% of the time.

CROSS-REACTIVITY

Samples containing 10.000 ng/mL Skeletal Troponin I, 2,000 ng/mL Troponin T, 1,390 ng/mL CK-MM, 1.000 ng/mL CK-BB and 20 mg/mL Cardiac Myosin have been tested with Myoglobin / CK-MB / Troponin I Test. No cross reactivity was observed, indicating that Myoglobin / CK-MB / Troponin I Test has a high degree of specificity for Myoglobin, CK-MB and Troponin I.

INTERFERING SUBSTANCES

Myoglobin/CK-MB/Troponin I Combo Test Device has been tested and no interference was observed in specimens containing 110 mg/mL human albumin, 6 mg/mL bilirubin, 10 mg/mL hemoglobin, 5 mg/mL cholesterol and 15 mg/mL triglycerides.
The following compounds have also been tested using the Myoglobin/CK-MB/Troponin I Combo Test Device and no interference was observed.

Acetaminophen
Acetoacetic Acid
Acetylsalicylic acid
Anisodamine
Ascorbic Acid
Atenolol
Atorvastatin Calcium
Caffeine
Captopril
Chloramphanicol
Chlordiazepoxide
Cilazapril
Creatine
Diclofenac
Digoxin
DL-Tyrosine
Ethanol
Felodipine.
Flunarizine Hydrochloride
Furosemide
Gentisic Acid
Hydrochlorothiazide
Isosorbide Mononitrate
Labetalol
Metoprolol Tartrate
Moraczine Hydrochloride
Nifedipine
Oxalic Acid
Oxazepam
Pentoxifyline
Phenobarbital
Quinine
Ramipril
Verapamil

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SYMBOLS USED

CE
Manufacturer
EC REP Authorized European Representative
Attention, see instruction for use
Consult instruction for use
IVD *In vitro* diagnostic medical device
Σ n Number of test
For single use only
Storage temperature
LOT Lot number
REF Catalog number
Expiry date