REFERENCES

INTENDED USE
The Myoglobin ELISA is intended for the quantitative determination of myoglobin in human serum. For research use only.

SUMMARY AND EXPLANATION
Myoglobin, a heme protein with a molecular weight of approximately 17,500 Daltons is found in both cardiac and skeletal muscle. Damage to either type of muscle following conditions such as trauma, ischemia, and diseases that cause myopathy, is associated with the release of myoglobin into serum. Specifically, following cardiac necrosis associated with myocardial infarction (MI), myoglobin is one of the first markers to rise above normal levels. Myoglobin levels increase measurably above baseline within 2-4 hours post-infarct, peaking at 9-12 hours, and returning to baseline within 24-36 hours. In the absence of skeletal muscle trauma or other factors associated with a non-cardiac related increase in circulating myoglobin, its levels have been used as an early marker for myocardial infarct. A number of reports suggest using the measurement of myoglobin as a diagnostic aid in ruling out myocardial infarction with negative predictive values of up to 100% reported at certain time periods after the onset of symptoms. 

PRINCIPLE OF THE TEST
The Myoglobin ELISA test is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes a unique monoclonal antibody directed against a distinct antigenic determinant on the myoglobin molecule. Mouse monoclonal anti-myoglobin antibody is used for solid phase immobilization on the microtiter wells. A goat anti-myoglobin antibody is used in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the two antibodies, resulting in the myoglobin molecules being sandwiched between the solid phase and enzyme-linked antibodies. After a 45 minute incubation at room temperature, the wells are washed with water to remove unbound labeled antibodies. A TMB (Tetramethyl-benzidine) Reagent is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of Stop Solution changing the color to yellow. The concentration of myoglobin is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

MATERIALS PROVIDED

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microwell coated with murine monoclonal anti-myoglobin.</td>
<td>12x8x1</td>
</tr>
<tr>
<td>Reference Standard Set</td>
<td>1 ml</td>
</tr>
<tr>
<td>Sample Diluent</td>
<td>25 ml</td>
</tr>
<tr>
<td>Enzyme Conjugate Reagent</td>
<td>22 ml</td>
</tr>
<tr>
<td>TMB Reagent</td>
<td>11 ml</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>11 ml</td>
</tr>
</tbody>
</table>

MATERIALS NOT PROVIDED

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450 nm
5. Absorbance paper or paper towel
6. Graph paper

Warning
All GenWay kits have not been tested for clinical use and are not approved in the United States by the FDA for diagnostic clinical use. They are components or reagents made solely for research use, further manufacturing and export use. It is the commitment of GenWay customers to receive its products solely for the purpose of exportation or research, and not for the purposes of clinical diagnostic use.

GENWAY BIOTECH, INC. DOES NOT MAKE ANY OTHER WARRANTY OR REPRESENTATION WHATSOEVER, WHETHER EXPRESS OR IMPLIED, WITH RESPECT TO THESE PRODUCTS. IN PARTICULAR GENWAY BIOTECH, INC. DOES NOT MAKE ANY WARRANTY OF SUITABILITY, NON-INFRINGEMENT, MERCHANTABILITY OR FITNESS FOR ANY PARTICULAR PURPOSE OF ANY PRODUCT.
STORAGE AND STABILITY
1. Store the kit at 2 – 8 ºC.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun or strong light.

WARNINGS AND PRECAUTIONS
1. Potential hazardous materials: The calibrator and controls contain human source components, which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984
2. This kit is designed for Research Use Only.
3. Optimal results will be obtained by strict adherence to the test protocol. Precise pipetting as well as following the exact time and temperature requirements is essential.
4. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
5. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
6. This product contains components preserved with sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azide. On disposal, flush with a large volume of water.

REAGENT PREPARATION
1. All reagents should be brought to room temperature (18-25°C) before use.
2. Patient serum and control serum should be diluted 10 fold before use. Prepare a series of small tubes (such as 1.5 ml microcentrifuge tubes) and mix 20 μl serum with 180 μl (0.18 ml) Sample Diluent. PLEASE DO NOT DILUTE THE STANDARDS – THEY HAVE ALREADY BEEN PRE-DILUTED 10-FOLD.
3. Samples with expected myoglobin concentrations over 1000 ng/ml may be quantitated by further dilution 10-fold with sample diluent.

ASSAY PROCEDURE
1. Patient serum and control serum should be diluted 10 fold before use. Prepare a series of small tubes (such as 1.5 ml microcentrifuge tubes) and mix 20 μl serum or plasma with 180 μl (0.18 ml) Sample Diluent. PLEASE DO NOT DILUTE THE STANDARDS – THEY HAVE ALREADY BEEN PRE-DILUTED 10-FOLD.
2. Secure the desired number of coated wells in the holder.
3. Dispense 20 μl of myoglobin standards, diluted specimens and diluted controls into the appropriate wells.
4. Dispense 200 μl of Enzyme Conjugate Reagent into each well.
5. Thoroughly mix for 30 seconds. It is very important to mix completely.
6. Incubate at room temperature (18-25°C) for 45 minutes.
7. Remove the incubation mixture by flicking plate contents into a waste container.
8. Rinse and flick the microwells 5 times with distilled or deionized water. (Please do not use tap water.)
9. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water drops.
10. Dispense 100 μl of TMB Reagent solution into each well. Gently mix for 5 seconds.
11. Incubate at room temperature for 20 minutes.
12. Stop the reaction by adding 100 μl of Stop Solution to each well.
13. Gently mix 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.
14. Read absorbance at 450 nm with a microtiter well reader within 15 minutes.

PERFORMANCE CHARACTERISTICS
Correlation coefficient = 0.9392, Slope = 0.9948, Intercept = 55.051, The Mean = 287.9 ng/ml, Abbott Myoglobin Mean = 262.5 ng/ml

1. SENSITIVITY
The lowest detectable level of myoglobin by this assay is estimated to be 5 ng/ml.

2. PRECISION
Intra-Assay Precision
Within-run precision was determined by replicate determinations of five different serum samples in one assay. Within-assay variability as shown:

<table>
<thead>
<tr>
<th>Sample</th>
<th># Reps.</th>
<th>Mean Myo. (ng/ml)</th>
<th>S.D.</th>
<th>C.V. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>5, 1, 6, 4, 5</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>2, 9, 3, 6</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>6, 5, 5, 5, 6</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>7, 5, 5, 5, 7</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>6, 5, 5, 5, 6</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

3. Inter-Assay Precision
Between-run precision was determined by replicate measurements of five different serum samples over a series of individually calibrated assays. Between-assay variability is shown below:

<table>
<thead>
<tr>
<th>Sample</th>
<th># Replicates</th>
<th>Mean Myo. (ng/ml)</th>
<th>S.D.</th>
<th>C.V. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>5, 1, 6</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>2, 9, 3, 6</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>12, 38, 52</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>7, 9, 7</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>9, 6, 7</td>
<td>9</td>
<td>9</td>
</tr>
</tbody>
</table>

4. RECOVERY
Various patient serum samples of known myoglobin levels were combined and assayed in duplicate. The mean recovery was 102.8%.