REFERENCES


2016-06-08

Myoglobin ELISA
Catalog No.: MBS580055 (96 Tests)

INTENDED USE
For Research Use Only. Not for use in diagnostic procedures.

MATERIALS PROVIDED

<table>
<thead>
<tr>
<th>Material Provided</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>0.5 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>
<tr>
<td>Wash Concentrate 20x: 1 Bottle</td>
<td>25 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

STORAGE AND STABILITY

1. Store the kit at 2 – 8°C.
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. For Research Use Only. Not for use in diagnostic procedures.
2. Potential biohazardous 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
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 (20-25°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.
4. Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (20-25°C).

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 (20-25°C) for 45 minutes.
7. Remove the incubation mixture by flicking plate contents into a waste container.
8. Remove liquid from all wells. Wash wells three times with 300 μl of 1X wash buffer. Blot on absorbance paper or paper towel.
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.

STANDARD CURVE

<table>
<thead>
<tr>
<th>CONCENTRATION (NG/ML)</th>
<th>O.D. 450 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.045</td>
</tr>
<tr>
<td>25</td>
<td>0.191</td>
</tr>
<tr>
<td>100</td>
<td>0.628</td>
</tr>
<tr>
<td>250</td>
<td>1.445</td>
</tr>
<tr>
<td>500</td>
<td>2.178</td>
</tr>
<tr>
<td>1000</td>
<td>2.896</td>
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