Lysozyme ELISA

The MyBioSource Lysozyme ELISA Kit is intended for the determination of lysozyme in human serum or stool.

Catalog Number: MBS494879 (96T)
Storage: 2-8°C
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1 INTRODUCTION

1.1 Intended Use
The MyBioSource Lysozyme ELISA Kit is intended for the determination of lysozyme in human serum or stool.

1.2 Summary and Explanation
Lysozyme (muramidase) is an enzyme present in serum, plasma, amniotic fluid, stool, saliva, tears, urine and other biological fluids. Elevated lysozyme levels in urine and serum have been reported in many human disease states, including Crohn’s disease, leukemias (FAB-M4, CMML, CML), tuberculosis, megaloblastic anemias, acute bacterial infections, ulcerative colitis, severe renal insufficiency, pyelonephritis, nephrosis and renal transplant rejection.

2 PRINCIPLE OF THE TEST
The MyBioSource Lysozyme kit is a solid phase direct ELISA sandwich method. The unknowns and the working anti-lysozyme enzyme conjugate are added to the wells coated with anti-lysozyme monoclonal antibody. Lysozyme in the unknown is bound to the monoclonal capture antibody and detected with a polyclonal detection antibody. Unbound lysozyme and anti-lysozyme enzyme conjugate is washed off by washing buffer. Upon the addition of the substrate, the intensity of color is proportional to the concentration of Lysozyme in the unknowns. A calibration curve is generated relating color intensity to the concentration of Lysozyme.

3 WARNINGS AND PRECAUTIONS
1. 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 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 Bio-medical Laboratories" 1984.

2. This test kit is a USA FDA exempt product.
3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which unknowns or kit reagents are handled.
4. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
5. It is recommended that calibrators, control and serum unknowns be run in duplicate.
6. Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

4 REAGENTS
4.1 Reagents provided

<table>
<thead>
<tr>
<th>MATERIALS PROVIDED</th>
<th>96 tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Microwell plate coated with anti-Lysozyme Monoclonal Ab</td>
<td>12x8x1</td>
</tr>
<tr>
<td>2. Lysozyme Calibrator: 7 vials (ready to use) Concentrations: 0, 1.25, 2.5, 5, 10, 20, 40 ng/mL</td>
<td>0.25ml</td>
</tr>
<tr>
<td>3. Lysozyme Controls: 2 vials (ready to use)</td>
<td>0.25ml</td>
</tr>
<tr>
<td>4. Anti-Lysozyme Enzyme Conjugate: 1 Vial (Ready to use)</td>
<td>12ml</td>
</tr>
<tr>
<td>5. Sample Diluent (ready to use)</td>
<td>40ml</td>
</tr>
<tr>
<td>6. TMB Substrate: 1 bottle (ready to use)</td>
<td>12ml</td>
</tr>
<tr>
<td>7. Stop Solution: 1 bottle (ready to use)</td>
<td>12ml</td>
</tr>
<tr>
<td>8. 20X Wash concentrate: 1 bottle</td>
<td>25ml</td>
</tr>
</tbody>
</table>
4.2 Materials required but not provided
1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel
6. Graph paper

4.3 Storage Conditions / Expiration
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.

4.4 Reagent Preparation
1. Wash Concentrate: 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 (18-26°C).

5 COLLECTION AND HANDLING OF UNKNOWNS

5.1 Preparation and Storage of Unknowns
1. Collect blood unknowns and separate the serum immediately.
2. Unknowns may be stored refrigerated at (2-8°C) for 5 days. If storage time exceeds 5 days, store frozen at (-20°C) for up to one month.
3. Avoid multiple freeze-thaw cycles.
4. Prior to assay, frozen sera should be completely thawed and mixed well.
5. Do not use grossly lipemic unknowns.

5.2 Sample Dilution Guideline

6 ASSAY PROCEDURE

6.1 General Remarks
1. For research use only.
2. Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

6.2 Test Procedure
Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (18-26°C).
1. Format the microplate wells for each serum reference, control and unknown to be assayed in duplicate. Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.
2. Pipette 25µl of the calibrators, controls and diluted unknowns into the assigned well.
3. Add 100µl of anti-lysozyme enzyme conjugate solution into all wells.
4. Incubate the plate for 60 minutes at room temperature, with shaking.
5. Remove liquid from all wells. Wash wells three times with 300 of 1X wash buffer (see Reagent Preparation Section). Blot on absorbent paper towels.
6. Add 100µl of TMB substrate solution to all wells
7. Incubate the plate for 15 minutes at room temperature.
8. Add 50µl of stop solution to each well and gently mix for 15-20 seconds.
9. Read the absorbance on ELISA Reader of each well at 450nm within 15 minutes after adding the stop solution.
6.3 Results

The calibration curve is constructed as follows:

1. Check Lysozyme calibrator value on each calibrator vial. This value might vary from lot to lot. Make sure you check the value on every kit.
2. To construct the calibration curve, plot the absorbance for Lysozyme calibrators (vertical axis) versus Lysozyme calibrator concentrations (horizontal axis) on a linear graph paper. Draw the best curve through the points.
3. Read the absorbance for controls and each unknown from the curve. Record the value for each control or unknown.

7 PERFORMANCE CHARACTERISTICS

7.1 Sensitivity

Sensitivity was determined by testing 20 negative samples and adding the mean plus two times the standard deviation of the results. The sensitivity is 0.021 ng/ml.

<table>
<thead>
<tr>
<th>Serum</th>
<th>Number of Replicates</th>
<th>Mean (ng/ml)</th>
<th>Standard Deviation</th>
<th>Sensitivity (Mean+2SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero Standard</td>
<td>20</td>
<td>0.002</td>
<td>0.010</td>
<td>0.021</td>
</tr>
</tbody>
</table>

8 REFERENCES / LITERATURE