Lysozyme ELISA
For the determination of lysozyme in serum, urine, and liquor.
For research use only, not for use in diagnostic procedures.

Catalog Number: MBS495103 (96T)
Storage: 2-8°C
1. **INTENDED USE**
This ELISA is intended for the determination of Lysozyme in human serum, urine and liquor. It is for research use only, not for use in diagnostic procedures.

2. **INTRODUCTION**
Lysozyme (muramidase) is a protein with a molecular weight of approx. 15 kDa and belongs to the group of alkaline glycosidases. Lysozyme is produced by granulocytes, monocytes and macrophages. The main source for faecal Lysozyme are the intestinal granulocytes. Lysozyme can be detected in all cells of the inflammatory infiltrate during an acute attack of Crohn’s disease. To some extent, Lysozyme is also secreted actively by mononuclear cells into the bowel lumen.

3. **MATERIAL SUPPLIED**

<table>
<thead>
<tr>
<th>Part #</th>
<th>Label</th>
<th>Kit components</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>K 6902MTP</td>
<td>PLATE</td>
<td>One holder with precoated strips</td>
<td>12 x 8 wells</td>
</tr>
<tr>
<td>K 6902WB</td>
<td>WASHBUF</td>
<td>ELISA wash concentrate 10 x</td>
<td>2 x 100 ml</td>
</tr>
<tr>
<td>K 6902VP</td>
<td>CONJBUF</td>
<td>Conjugate dilution buffer</td>
<td>1 x 15 ml</td>
</tr>
<tr>
<td>K 6902K</td>
<td>CONJ</td>
<td>Conjugate concentrate, (rabbit-anti-Lysozyme, Peroxidase-labeled)</td>
<td>1 x 50 µl</td>
</tr>
<tr>
<td>K 6902ST</td>
<td>STD</td>
<td>Standards, ready to use (0; 1,1 ;3,3; 10; 30 ng/ml)</td>
<td>5 x 1 ml</td>
</tr>
<tr>
<td>K 6902KO1</td>
<td>CTRL</td>
<td>Control, ready to use</td>
<td>1 x 1 ml</td>
</tr>
<tr>
<td>K 6902KO2</td>
<td>CTRL</td>
<td>Control, ready to use</td>
<td>1 x 1 ml</td>
</tr>
<tr>
<td>K 6902TMB</td>
<td>SUB</td>
<td>TMB substrate (Tetramethylbenzidine)</td>
<td>1 x 15 ml</td>
</tr>
<tr>
<td>K 6902AC</td>
<td>STOP</td>
<td>ELISA stop solution, ready to use</td>
<td>1 x 15 ml</td>
</tr>
</tbody>
</table>
4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultra pure water*
- Laboratory balance
- Calibrated precision pipettors and 10–1000 µl tips
- Foil to cover the microtiter plate
- Horizontal microtiter plate shaker
- Multi-channel pipets or repeater pipets
- Centrifuge, 3000 g
- Vortex
- Standard laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader (required filters see chapter 7)

* MyBioSource recommends the use of Ultra Pure Water (Water Type 1: ISO 3696), which is free of undissolved and colloidal ions and organic molecules (free of particles > 0.2 µm) with an electrical conductivity of 0.055 µS/cm at 25 °C (≥ 18.2 MΩ cm).

5. STORAGE AND PREPARATION OF REAGENTS

- To run the assay more than once, ensure that reagents are stored at the conditions stated on the label. **Prepare only the appropriate amount necessary for each run.** The kit can be used up to 4 times within the expiry date stated on the label.

- Reagents with a volume less than 100 µl should be centrifuged before use to avoid loss of volume.

- The **ELISA wash buffer concentrate** (WASHBUF) should be diluted **1:10 in ultra pure water** before use (100 ml concentrate + 900 ml ultra pure water), mix well. Crystals could occur due to high salt concentration in the stock solutions. The crystals must be redissolved at room temperature or 37 °C before dilution of the buffer solutions. The **buffer concentrate** is stable at 2–8 °C until the expiry date stated on the label. **Diluted buffer solution** (wash buffer) can be stored in a closed flask at 2–8 °C for one month.

- The **conjugate concentrate** (CONJ) must be diluted **1:1000 in conjugate dilution buffer** (100 µl CONJ + 10 ml CONJBUF). The concentrate is stable at 2–8 °C until the expiry date stated on the label. **Diluted conjugate is not stable and cannot be stored.**

- All other test reagents are ready to use. Test reagents are stable until the expiry date (see label of test package) when stored at 2–8 °C.

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- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES-
6. STORAGE AND PREPARATION OF SAMPLES

Serum
Serum should be centrifuged within one hour after collection. Store samples at -20°C if not assayed on the same day. Lipemic or hemolytic samples may give false results. Samples should be mixed well before assaying. Samples are diluted between 1:500 and 1:1000 with wash buffer.

Use this dilution factor to calculate the Lysozyme concentration.

Urine
We recommend a dilution of 1:5 with wash buffer for the urine samples before analysis.

Liquor
We recommend a dilution of 1:50 in wash buffer for the liquor samples before analysis.

7. ASSAY PROCEDURE

Principle of the test
The assay utilizes the “sandwich” technique with two selected antibodies that recognize human lysozyme.

Standards, controls and diluted samples, which are assayed for human lysozyme, are added into the wells of a micro plate coated with a high affinity anti-human lysozyme antibody. During the first incubation step, lysozyme is bound by the immobilized antibody. Then a peroxidase-conjugated anti-human lysozyme antibody is added into each microtiter well and a “sandwich” of capture antibody - human lysozyme – peroxidase-conjugate is formed. Tetramethylbenzidine is used as peroxidase substrate. Finally, an acidic stop solution is added to terminate the enzymatic reaction. The color changes from blue to yellow. The intensity of the yellow color is directly proportional to the concentration of lysozyme. A dose response curve of the absorbance unit (optical density, OD at 450 nm) vs. concentration is generated using the values obtained from the standards. Lysozyme present in the samples is determined directly from this curve.
**Test procedure**

Bring all **reagents and samples to room temperature** (15–30 °C) and mix well. Take as many **microtiter strips** as needed from kit. Store unused strips covered at 2-8°C. Strips are stable until expiry date stated on the label. We recommend to carry out the tests in duplicate.

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Mark the positions of <strong>STD/SAMPLE/CTRL</strong> (standard/sample/controls) on a protocol sheet</td>
</tr>
<tr>
<td>2.</td>
<td>Wash the wells 5x with <strong>250 µl of diluted WASHBUF</strong> (Wash buffer), remove remaining WASHBUF by hitting the plate against paper towel after the last wash</td>
</tr>
<tr>
<td>3.</td>
<td>Add <strong>100 µl of STD/SAMPLE/CTRL</strong> (standard/sample/controls) into respective well</td>
</tr>
<tr>
<td>4.</td>
<td>Cover the plate tightly and incubate for <strong>1 hour</strong> at room temperature (15-30 °C) on a horizontal mixer</td>
</tr>
<tr>
<td>5.</td>
<td>Aspirate and wash the wells 5x with <strong>250 µl of diluted WASHBUF</strong> (Wash buffer), remove remaining WASHBUF by hitting the plate against paper towel after the last wash</td>
</tr>
<tr>
<td>6.</td>
<td>Add <strong>100 µl diluted CONJ</strong> (Conjugate) into each well</td>
</tr>
<tr>
<td>7.</td>
<td>Cover the plate tightly and incubate for <strong>1 hour</strong> at room temperature (15-30 °C) on a horizontal mixer</td>
</tr>
<tr>
<td>8.</td>
<td>Aspirate and wash the wells 5x with <strong>250 µl of diluted WASHBUF</strong> (Wash buffer), remove remaining WASHBUF by hitting the plate against paper towel after the last wash</td>
</tr>
<tr>
<td>9.</td>
<td>Add <strong>100 µl of SUB</strong> (Substrate) into each well</td>
</tr>
<tr>
<td>10.</td>
<td>Incubate for <strong>10 - 20 min.</strong>* at room temperature (15-30 °C) in the dark</td>
</tr>
<tr>
<td>11.</td>
<td>Add <strong>100 µl of STOP</strong> (Stop solution) into each well, mix well</td>
</tr>
<tr>
<td>12.</td>
<td>Determine <strong>absorption immediately</strong> with an ELISA reader at <strong>450 nm</strong> against 620 nm (or 690 nm) as a reference. If no reference wavelength is available, read only at 450 nm. If the extinction of the highest standard exceeds the range of the photometer, absorption must be measured immediately at <strong>405 nm</strong> against 620 nm as a reference.</td>
</tr>
</tbody>
</table>

* The intensity of the color change is temperature sensitive. We recommend observing the color change and stopping the reaction upon good differentiation.
8. RESULTS
The following algorithms can be used alternatively to calculate the results. We recommend using the “4 parameter algorithm”.

1. **4 parameter algorithm**
   It is recommended to use a linear ordinate for the optical density and a logarithmic abscissa for the concentration. When using a logarithmic abscissa, the zero standard must be specified with a value less than 1 (e.g. 0.001).

2. **Point-to-point calculation**
   We recommend a linear ordinate for the optical density and a linear abscissa for the concentration.

3. **Spline algorithm**
   We recommend a linear ordinate for the optical density and a linear abscissa for the concentration.

The plausibility of the duplicate values should be examined before the automatic evaluation of the results. If this option is not available with the program used, the duplicate values should be evaluated manually.

**Serum**
The result should be multiplied by the corresponding dilution factor to obtain the serum value.

**Urine**
The estimated values are multiplied by a dilution factor of 5.

**Liquor**
The estimated values are multiplied by a dilution factor of 50.

In case another dilution factor has been used, multiply the obtained result with the dilution factor used.

9. LIMITATIONS
Samples with concentrations above the measurement range must be further diluted and re-assayed. Please consider this greater dilution when calculating the results.

Samples with concentrations lower than the measurement range cannot be clearly determined.

The upper limit of the measurement range can be calculated as:

\[
\text{highest concentration of the standard curve} \times \text{sample dilution factor to be used}
\]
The lower limit of the measurement range can be calculated as:

\[
\text{detection limit} \times \text{sample dilution factor to be used}
\]

### 10. QUALITY CONTROL

MyBioSource recommends the use of external controls for internal quality control, if possible.

Control samples should be analyzed with each run. Results, generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods. The results for the samples may not be valid if within the same assay one or more values of the quality control sample are outside the acceptable limits.

**Reference range**

**Serum***

700 - 2580 ng/ml

The lysozyme concentration depends on its production by monocytes, macrophages, granulocytes as well as kidney parenchyma cells.

Lysozyme is elevated in: myelomonocytic leukosis, sarcoidosis

Lysozym is reduced in: newborn sepsis, panmyelopathy

**Urine***

1.7 - 123 ng/ml

Lysozyme in urine is elevated in: myelomonocytic leukemia, urinary passage infection of children

Lysozyme in urine is reduced in: panmyelopathy

**Liquor***

< 62 ng/ml

* Labor Dr. Limbach, Heidelberg, http://www.labor-limbach.de

We recommend each laboratory to establish its own reference range.
11. PERFORMANCE CHARACTERISTICS

Analytical Sensitivity
The Zero-standard was measured 21 times. The detection limit was set as $B_0 + 1.645 \times SD$ and estimated to be 0.144 ng/ml.

Precision and reproducibility

Intra-Assay (n = 26)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Lysozyme [ng/ml]</th>
<th>CV [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>658.4</td>
<td>2.2</td>
</tr>
<tr>
<td>B</td>
<td>397.9</td>
<td>2.8</td>
</tr>
</tbody>
</table>

Inter-Assay (n = 18)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Lysozyme [ng/ml]</th>
<th>CV [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>696.5</td>
<td>4.6</td>
</tr>
<tr>
<td>B</td>
<td>391.4</td>
<td>6.4</td>
</tr>
</tbody>
</table>

Spiking Recovery
Two samples were spiked with different lysozyme concentrations and measured using this assay (n = 2).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Unspiked Sample</th>
<th>Spike [ng/ml]</th>
<th>Lysozyme expected [ng/ml]</th>
<th>Lysozyme measured [ng/ml]</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.46</td>
<td>1.8</td>
<td>2.26</td>
<td>2.17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.7</td>
<td>3.16</td>
<td>3.21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.0</td>
<td>5.46</td>
<td>5.51</td>
</tr>
<tr>
<td>B</td>
<td>0.74</td>
<td>1.8</td>
<td>2.54</td>
<td>2.48</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.7</td>
<td>3.44</td>
<td>3.50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.7</td>
<td>4.44</td>
<td>4.65</td>
</tr>
</tbody>
</table>
**Dilution recovery**
A sample was diluted and analyzed. The results are shown below (n = 1):

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dilution</th>
<th>Lysozyme expected [ng/ml]</th>
<th>Lysozyme measured [ng/ml]</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1:500</td>
<td>626.3</td>
<td>626.3</td>
</tr>
<tr>
<td></td>
<td>1:1000</td>
<td>313.2</td>
<td>324.2</td>
</tr>
<tr>
<td></td>
<td>1:2000</td>
<td>156.6</td>
<td>173.0</td>
</tr>
</tbody>
</table>

**Specificity**
No cross reactivity to other plasma proteins was observed.

12. **PRECAUTIONS**
- All reagents in the kit package are for research use only, not for use in diagnostic procedures.
- Human materials used in kit components were tested and found to be negative for HIV, Hepatitis B and Hepatitis C. However, for safety reasons, all kit components should be treated as potentially infectious.
- Kit reagents contain sodium azide or ProClin as bactericides. Sodium azide and ProClin are toxic. Substrates for the enzymatic color reactions are toxic and carcinogenic. Avoid contact with skin or mucous membranes.
- The stop solution consists of diluted sulphuric acid, a strong acid. Although diluted, it still must be handled with care. It can cause burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spill should be wiped up immediately with copious quantities of water. Do not breathe vapor and avoid inhalation.

13. **TECHNICAL HINTS**
- Do not interchange different lot numbers of any kit component within the same assay. Furthermore we recommend not assembling wells of different microtiter plates for analysis, even if they are of the same batch.
- Control samples should be analyzed with each run.
- Reagents should not be used beyond the expiration date stated on kit label.
- Substrate solution should remain colorless until use.
• To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
• Avoid foaming when mixing reagents.
• Do not mix plugs and caps from different reagents.
• The assay should always be performed according the enclosed manual.

14. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

• This assay was produced and distributed according to the guidelines of 98/79/EC.
• The guidelines for medical laboratories should be followed.
• Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test. MyBioSource can therefore not be held responsible for any damage resulting from incorrect use.
• Warranty claims and complaints regarding deficiencies must be logged within 14 days after receipt of the product. The product should be sent to MyBioSource along with a written complaint.

15. REFERENCES


