Arbeitsanleitung/Manual

Lysozym ELISA Kit

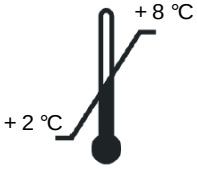
Zur in vitro Bestimmung des Lysozym aus Serum, Urin und Liquor

Lysozyme ELISA Kit

For the in vitro determination of Lysozyme in serum, urine and liquor

Gültig ab/valid from 25.10.2012

REF **K 6902**



IVD CE

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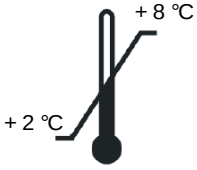

1. INTENDED USE

This ELISA is intended for the quantitative determination of Lysozyme in human serum, urine and liquor. It is for *in vitro* diagnostic use only.

2. SUMMARY AND EXPLANATION OF THE TEST

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.

Indication

- Diagnosis and monitoring of Crohn's Disease
- Early diagnosis of rejection reactions in kidney transplantation cases
- Differential diagnosis and monitoring of leukosis
- Diagnosis and treatment monitoring of urinary tract infections in children
- Differential diagnosis between viral and bacterial meningitis in children
- Identification of sepsis in neonates

3. MATERIAL SUPPLIED

Catalogue No	Content	Kit Components	Quantity
K 6902MTP	PLATE	one holder with precoated strips	12 x 8 wells
K 6902WB	WASHBUF	ELISA wash concentrate 10x	2 x 100 ml
K 6902VP	CONJBUF	Conjugate dilution buffer	1 x 15 ml
K 6902K	CONJ	Conjugate, (Rabbit-anti-Lysozyme, Peroxidase-labelled)	1 x 50 µl
K 6902ST	STD	Calibrators, ready to use (0; 1.1; 3.3; 10; 30 ng/ml)	5 x 1 ml
K 6902KO1	CTRL	Control, ready to use	1 x 1 ml
K 6902KO2	CTRL	Control, ready to use	1 x 1 ml
K 6902TMB	SUB	TMB substrate (Tetramethylbenzidine)	1 x 15 ml
K 6902AC	STOP	ELISA stop solution, ready to use	1 x 15 ml

4 MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultra pure water*
- Laboratory balance
- Precision pipettors calibrated to deliver 10-1000 µl
- Covering foil for the microtiter plate
- Horizontal microtiter plate shaker
- Multi-channel dispenser or repeating dispenser
- Centrifuge capable of 3000 x g
- Vortex-Mixer
- Standard laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader 450 nm

*Immundiagnostik AG 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 < 0.055 µS/cm at 25°C (≥18.2 MΩ cm).

5. PREPARATION AND STORAGE OF REAGENTS

- To run the assay more than one time, make sure that the reagents are carefully stored at 2-8°C. Prepare just the appropriate amount necessary for the assay. The kit can be used up to 4 times within the expiry date stated on the label.

- Reagents with a volume less then 100 µl should be centrifuged before use to avoid losses.

- The **ELISA wash buffer concentrate** (WASHBUF) should be diluted with ultra pure water **1:10** before use (100 ml WASHBUF + 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 at 37°C using a water bath 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** can be stored in a closed flask at **2-8°C for one month**.

- The **Conjugate** (CONJ, POD antibody) must be diluted **1:1000** in conjugate dilution buffer (10 µl CONJ and 10 ml CONJBUF). The antibody is stable at 2-8 °C until expiry date given on the label. **Diluted antibody solution is <u>not</u> stable and can not be stored.**

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

6. SAMPLE PREPARATION

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. We recommend that STD, CTRL and SAMPLE are assayed in duplicate..

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 in 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 affine 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 (TMB) is used as peroxidase substrate. Finally, an acidic stop solution is added to terminate the enzymatic reaction. The colour changes from blue to yellow. The intensity of the yellow colour 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

Prior to use in the assay allow all reagents and samples to come to room temperature and mix by gentle swirling and inversion.
Mark the positions of STD (Standards)/SAMPLE/CTRL (Controls) on a protocol sheet
Take microtiter strips out of the kit. Store unused strips covered at 2-8° C. Strips are stable until the expiry date stated on the label
Wash the wells 5x with 250 µl of diluted WASHBUF (Wash buffer), remove remaining WASHBUF by hitting the plate against paper towel after the last wash
Add 100 µl of STD/SAMPLE/CTRL (Standard/Sample/Controls) in duplicate into respective well
Cover the plate tightly and incubate for 1 hour at room temperature (18-26°C) on a horizontal mixer
Aspirate and wash the wells 5x with 250 µl of diluted WASHBUF (Wash buffer), remove remaining WASHBUF by hitting the plate against paper towel after the last wash
Add 100 µl CONJ (Conjugate) into each well
Cover the plate tightly and incubate for 1 hour at room temperature (18-

26°C) on a horizontal mixer
Aspirate and wash the wells 5x with 250 µl of diluted WASHBUF (Wash buffer), remove remaining WASHBUF by hitting the plate against paper towel after the last wash
Add 100 µl of SUB (Substrate) into each well
Incubate for 10 - 20 minutes at room temperature (18-26°C) in the dark
Add 50 µl of STOP (Stop solution) into each well, shake well
Determine the absorption with an ELISA reader at 450 nm 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 405 nm against 620 nm as a reference

8. RESULTS

The following algorithms can be used alternatively to calculate the results. However, we recommend to use the option 1: 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 calibrator has to be specified with a value smaller than 1 (e. g. 0.01).

2. Point-to-point-calculation

   We recommend for the optical density a linear ordinate and for the concentration a linear abscissa.

3. Spline-algorithm

   We recommend for the optical density a linear ordinate and for the concentration a logarithmic abscissa. When using a logarithmic abscissa, the zero calibrator has to be specified with a value smaller than 1 (e. g. 0.01).

   The plausibility of the pairs of values should be examined before the automatic evaluation of the results. If this option is not available with the used program, a manual control of the paired values should be made.

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.**

9. LIMITATIONS

Samples with Lysozyme value greater than the highest calibrator should be diluted and re-assayed.

10. QUALITY CONTROL

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. If one or more of the quality sample values are outside the acceptable limits, the results of the patient samples may be invalid.

Expected values

Reference ranges

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

It is recommended for each laboratory to establish its own normal range.

11. PERFORMANCE CHARACTERISTICS

Precision and reproducibility

Precision and reproducibility

Intra-Assay (n=26)		
Sample	Lysozyme [ng/ml]	Vk [%]
1	658.4	2.2
2	397.9	2.8

Inter-Assay (n=18)		
Sample	Lysozyme [ng/ml]	Vk [%]
1	696.5	4.6
2	391.4	6.4

Recovery

Two samples were spiked with Lysozyme calibrator and measured using this assay.

Recovery n=2

Sample [ng/ml]	Spike [ng/ml]	Lysozyme expected [ng/ml]	Lysozyme measured [ng/ml]
0.46	1.8	2.26	2.17
0.46	2.7	3.16	3.21
0.46	5.0	5.46	5.51
0.74	1.8	2.54	2.48
0.74	2.7	3.44	3.50
0.74	3.7	4.44	4.65

Sensitivity

The sensitivity limit (LoB) was set as B_0 + 1.645*SD. The zero-standard was measured 21 times.

Sample	Lysozyme mean value [OD]	Standard variation	Detection limit [ng/ml]
1	0.039	0.005	0.144

Cross reactivity

No cross reactivity to other plasma proteins was observed.

Sample dilution

n= 1

Sample	Dilution	Expected [ng/ml]	Measured [ng/ml]
A	1:500	626.3	626.3
	1:1000	313.2	324.2
	1:2000	156.6	173.0

12. PRECAUTIONS

- For *in vitro* diagnostic use only.
- The quality control guidelines should be observed.
- Human material used in the kit components was tested and found to be negative for HIV, Hepatitis B and Hepatitis C. However, all kit components should be treated as potentially infectious.
- Reagents of the kit package contain sodium azide and thimerosal as bactericides. Sodium azide and thimerosal are toxic. The substrates for the enzymatic color reactions are described to be also toxic and carcinogenic. Contact with skin or mucous membranes has to be avoided.
- Stop solution consists of sulfuric 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 out immediately with copious quantities of water.

13. TECHNICAL HINTS

- Do not interchange different lot numbers of any kit component within the same assay. Furthermore, we recommend not to assemble wells of different microtiter plates for analysis, even if they are of the same batch.
- Reagents should not be used beyond the expiration date shown on the kit label.
- Substrate solution should remain colourless until added to the plate.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Avoid foaming when mixing reagents.
- The assay should always be performed according the enclosed manual.
- Incubation time, incubation temperature and the volumes of the different components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the test results. Immundiagnostik can therefore not be held responsible for any damage resulting from this.

14. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- This assay was produced and put on the market according to the IVD guidelines of 98/79/EC.
- The kit components made of human serum are tested for Hepatitis B, Hepatitis C and HIV and found to be negative. However, since no test method can offer complete assurance that infectious agents are absent, these reagents should be handled as recommended for any potentially infectious human serum or blood specimen. The normal precautions for laboratory work should be observed.
- All reagents in the kit package are for *in vitro* diagnostic use only.
- The guidelines for medical laboratories should be observed.
- Incubation time, incubation temperature and volumes of the different 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. Immundiagnostik can therefore not be held responsible for any damage resulting from this.

- Warranty claims and complaints in respect of deficiencies must be lodged within 14 days of receipt of the product. The product shall be send to Immundiagnostik together with the complaint in writing.

15. REFERENCES

1. Hemrika et al.: 1989; Neth. J. Med. 34, 174
2. Arndt et al.: 1993, Crohn. Klein. Lab. 11, 867
3. Stein, J. et al.: 1996; 3. Post-Graduiertenkurs der DGVS

Used symbols: