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Manual

Lysozyme ELISA

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

Valid from 2020-09-15

REF **K 6902**

Σ 96

+8°C +2°C

IVD

CE

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1. INTENDED USE

This Immundiagnostik AG assay is an enzyme immunoassay intended for the quantitative determination of lysozyme in human serum, urine and liquor. For *in vitro* diagnostic use only.

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.

Indications

- 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

Cat. No.	Label	Kit components	Quantity
K 6902	PLATE	Microtiter plate, pre-coated	12 x 8 wells
K 0001.C.100	WASHBUF	Wash buffer concentrate, 10 x	2 x 100 ml
K 6902	CONJBUF	Conjugate dilution buffer, ready-to-use	2 x 15 ml
K 6902	CONJ	Conjugate concentrate, (rabbit-anti-lysozyme, peroxidase-labelled)	1 x 50 µl
K 6902	STD	Standards, ready-to-use (0; 1.1; 3.3; 10; 30 ng/ml)	5 x 1 ml
K 6902	CTRL1	Control, ready-to-use (see specification for range)	1 x 1 ml
K 6902	CTRL2	Control, ready-to-use (see specification for range)	1 x 1 ml
K 6902	SAMPLEBUF	Sample dilution buffer, ready-to-use	1 x 50 ml

Cat. No.	Label	Kit components	Quantity
K 0002.15	SUB	Substrate (Tetramethylbenzidine)	1 x 15 ml
K 0003.15	STOP	Stop solution, ready-to-use	1 x 15 ml

For reorders of single components, use the catalogue number followed by the label as product number.

4. MATERIAL REQUIRED BUT NOT SUPPLIED

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

* Immundiagnostik AG recommends the use of ultrapure 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.
- **Preparation of the wash buffer:** The **wash buffer concentrate (WASHBUF)** has to be diluted with ultrapure water **1:10** before use (100 ml WASHBUF + 900 ml ultrapure water), mix well. Crystals could occur due to high salt concentration in the concentrate. Before dilution, the crystals have to be redissolved at room temperature or in a water bath at 37 °C. The **WASHBUF** can be used until the expiry date stated on the label when stored at **2–8 °C**. **Wash buffer** (1:10 diluted WASHBUF) can be stored in a closed flask at **2–8 °C for 1 month**.

- **Preparation of the conjugate:** Before use, the **conjugate concentrate (CONJ)** has to be diluted **1:1001** in **conjugate dilution buffer (CONJBUF)** (10 µl CONJ + 10 ml CONJBUF). The **CONJ** can be used until the expiry date stated on the label when stored at **2–8 °C**. **Conjugate** (1:1001 diluted CONJ) **is not stable and cannot be stored.**
- All other test reagents are ready-to-use. Test reagents can be used until the expiry date (see label) when stored at **2–8 °C**.

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.

Lysozyme should be determinated within **8 hours,** after this time the concentration decreases at **room temperature**. The sample can be stored **6 days at 4 °C**.*

Lipemic or hemolytic samples may give false results. Samples should be mixed well before assaying. Samples are diluted between **1:500** and **1:1 000** with wash buffer.

Use this dilution factor to calculate the lysozyme concentration.

*Thomas, L., 1998, Labor und Diagnose, 5th ed. TH-Books, Frankfurt.

Urine

We recommend a dilution of **1:5** with sample dilution 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 utilises the "sandwich" technique with two selected antibodies that recognise human lysozyme.

Standards, controls and diluted samples, which are assayed for human lysozyme, are added into the wells of a microtiter plate coated with a high affine anti-human lysozyme antibody. During the first incubation step, lysozyme is bound by the im-

mobilised 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 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

Bring all **reagents and samples to room temperature** (15–30 °C) and mix well.

Mark the positions of standards/controls/samples on a protocol sheet.

Take as many microtiter strips as needed from the kit. Store unused strips together with the desiccant bag in the closed aluminium packaging at 2–8 °C. Strips are stable until the expiry date stated on the label.

For automated ELISA processors, the given protocol may need to be adjusted according to the specific features of the respective automated platform. For further details please contact your supplier or Immundiagnostik AG.

We recommend to carry out the tests in duplicate.

1.	**Before use**, wash the wells **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
2.	Add each **100 µl standards/controls/samples** into the respective wells.
3.	Cover the strips and incubate for **1 hour** at room temperature (15–30 °C) on a **horizontal shaker***.
4.	Discard the content of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
5.	Add **100 µl conjugate** (diluted CONJ) into each well.
6.	Cover the strips and incubate for **1 hour** at room temperature (15–30 °C) on a **horizontal shaker***.
7.	Discard the content of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.

8.	Add **100 µl substrate** (SUB) into each well.
9.	Incubate for **10–20 min**** at room temperature (15–30 °C) in the **dark**.
10.	Add **100 µl stop solution** (STOP) into each well and mix well.
11.	Determine **absorption immediately** 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.

* We recommend shaking the strips at 550 rpm with an orbit of 2 mm.

** The intensity of the colour change is temperature sensitive. We recommend observing the colour 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 programme used, the duplicate values should be evaluated manually.

Serum

The obtained results have to be multiplied by the **corresponding dilution factor** to get the actual concentrations.

Urine

The obtained results have to be multiplied by the **dilution factor of 5** to get the actual concentrations.

Liquor

The obtained results have to be multiplied by the **dilution factor of 50** to get the actual concentrations.

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

9. LIMITATIONS

Samples with concentrations above the measurement range (see definition below) can be further diluted and re-assayed. Please consider this higher dilution when calculating the results.

Samples with concentrations lower than the measurement range (see definition below) cannot be clearly quantified.

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

highest concentration of the standard curve × *sample dilution factor to be used*

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

LoB × *sample dilution factor to be used*

LoB see chapter "Performance Characteristics".

10. QUALITY CONTROL

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

Control samples should be analysed with each run. Results, generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods. The results for the patient 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*

700–2 580 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

Accuracy - Precision

Repeatability (Intra-Assay); n = 26

The repeatability was assessed with 3 serum samples under **constant** parameters (same operator, instrument, day and kit lot).

Sample	Mean value [ng/ml]	CV [%]
1	658.36	2.2
2	397.90	2.8
3	658.50	3.9

Reproducibility (Inter-Assay)

The reproducibility was assessed with 2 serum samples under **varying** parameters (different operators, instruments, days and kit lots).

Sample	Mean value [ng/ml]	CV [%]
1 (n = 47)	692.82	6.8
2 (n = 35)	386.62	6.6

Accuracy - Trueness

The trueness states the closeness of the agreement between the result of a measurement and the true value of the measurand. Therefore, Lysozyme-spikes with known concentrations were added to 4 serum samples. The table below shows the results without consideration of the sample dilution factor:

Sample [ng/ml]	Spike [ng/ml]	Expected [ng/ml]	Obtained [ng/ml]	Recovery [%]
0.74	2.22	2.91	2.71	93.33
	2.73	3.40	3.24	95.18
	3.21	3.88	3.50	90.27
	4.14	4.78	4.65	97.45
	5.00	5.62	6.01	106.91
0.46	2.22	2.65	2.17	81.92
	3.21	3.62	3.21	88.67
	4.14	4.53	4.73	104.42
	5.00	5.38	5.51	102.36
1.25	2.22	3.38	3.16	93.37
	3.21	4.33	3.47	79.95
	4.14	5.22	4.58	87.68
	5.00	6.05	5.58	92.34
0.65	2.22	2.82	2.38	84.38
	3.21	3.79	3.48	91.58
	4.14	4.70	4.25	90.46
	5.00	5.54	5.10	92.03

Linearity

The linearity states the ability of a method to provide results proportional to the concentration of analyte in the test sample within a given range. This was assessed according to CLSI guideline EP06-A by serial dilution of 2 serum- and 2 urine-samples.

For Lysozyme in serum and urine, the method has been demonstrated to be linear from 0.71 to 11.29 ng/ml, showing a non-linear behaviour of less than ± 20 % in this interval without consideration of the sample dilution factor:

Sample	Dilution	Expected [ng/ml]	Obtained [ng/ml]	Recovery [%]
Serum 1	1:500	5.87	5.87	100.00
	1:1 000	2.94	2.75	93.57
	1:2 000	1.47	1.51	102.82
	1:4 000	0.73	0.89	121.46
Serum 2	1:500	6.46	6.46	100.00
	1:1 000	3.23	2.96	91.62
	1:2 000	1.62	1.51	93.73
	1:4 000	0.81	0.84	103.57
Urine 1	1:5	11.29	11.29	100.00
	1:10	5.65	5.91	104.57
	1:20	2.82	2.74	97.16
	1:40	1.41	1.41	99.73
	1:80	0.71	0.75	106.72
Urine 2	1:5	10.42	10.42	100.00
	1:10	5.21	3.85	73.77
	1:20	2.61	1.82	70.01
	1:40	1.30	1.06	81.52

Analytical sensitivity

The following values have been estimated based on the concentrations of the standard without considering possibly used sample dilution factors.

Limit of blank, LoB	0.615 ng/ml
Limit of detection, LoD	0.821 ng/ml
Limit of quantitation, LoQ	0.821 ng/ml

The evaluation was performed according to the CLSI guideline EP-17-A2. The specified accuracy goal for the LoQ was 20 % CV.

Analytical specificity

The specificity of the antibody was tested by measuring the cross-reactivity against compounds with structural similarity to Lysosyme. There was no cross-reactivity observed with the following substances:

Substance tested	Concentration added	Conclusion
α1-Antitrypsin	90 µg/l	< LoB
Albumin	800 µg/l	< LoB
PMN elastase	40 ng/ml	< LoB
sIgA	600 ng/ml	< LoB
Human hemoglobin	1 000 µg/ml	< LoB
CRP	150 ng/ml	< LoD
Pancreatic amylase	28 333 mU/l	< LoB
Chymotrypsin	1 000 ng/ml	< LoB
IgE	500 ng/ml	< LoB
Hemoglobin-haptoglobin complex	40 mU/l	< LoB

12. PRECAUTIONS

- All reagents in the kit package are for *in vitro* diagnostic use only.
- 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 colour 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 breath vapour 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 analysed with each run.
- Reagents should not be used beyond the expiration date stated on kit label.
- Substrate solution should remain colourless 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 to the enclosed manual.

14. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- This assay was produced and distributed according to the IVD 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. Immundiagnostik AG 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 send to Immundiagnostik AG along with a written complaint.

15. REFERENCES

1. Langhorst, Jost, Sigrid Elsenbruch, Twyla Mueller, Andreas Rueffer, Guenther Spahn, Andreas Michalsen, and Gustav J Dobos. 2005. "Comparison of 4 Neutrophil-Derived Proteins in Feces as Indicators of Disease Activity in Ulcerative Colitis." *Inflammatory Bowel Diseases* **11** (12) (December): 1085–91.
2. Pettersson, C, H Karlsson, M Ståhlman, T Larsson, B Fagerberg, M Lindahl, O Wiklund, J Borén, and L Fogelstrand. 2011. "LDL-Associated Apolipoprotein J and Lysozyme Are Associated with Atherogenic Properties of LDL Found in Type 2 Diabetes and the Metabolic Syndrome." *Journal of Internal Medicine* **269** (3) (March): 306–21. doi:10.1111/j.1365-2796.2010.02292.x.
3. Schwaab M., Euteneuer M., Lautermann J., Sudhoff H. 2005. "Lysozym Und Laktoferrin in Adenoiden, Hyperplastischen Und Chronisch Entzündeten Tonsillen - Eine Quantitative Analyse." *Laryngo-Rhino-Otologie* **84**: 660–664. doi:10.1055/s.
4. Volman, Julia J, Ronald P Mensink, Wim a Buurman, and Jogchum Plat. 2011. "In Vivo Effects of Dietary (1→3), (1→4)-B-D-Glucans from Oat on Mucosal Immune Responses in Man and Mice." *Scandinavian Journal of Gastroenterology* **46** (5) (May): 603–10. doi:10.3109/00365521.2010.545830.
5. West, N P, D B Pyne, J M Kyd, G M Renshaw, P A Fricker, and A W Cripps. 2010. "The Effect of Exercise on Innate Mucosal Immunity." *British Journal of Sports Medicine* **44** (4) (March): 227–31. doi:10.1136/bjsm.2008.046532.

Used symbols:

 Temperature limitation

REF Catalogue Number

IVD In Vitro Diagnostic Medical Device

→REF To be used with

 Manufacturer

 Contains sufficient for <n> tests

LOT Lot number

 Use by

 Attention

 Consult instructions for use

 Consult specification data sheet