Manual

IDK® PMN-Elastase ELISA

For the determination of PMN elastase in serum, plasma, and seminal plasma

Gültig ab / Valid from 2018-01-15

REF K 6841

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+8 °C
+2 °C

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For further information about this kit, its application or the procedures in this kit insert, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.

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Table of Contents

1. INTENDED USE

This ELISA is intended for the quantitative determination of PMN elastase in serum, plasma and seminal plasma. It is for research use only.

2. INTRODUCTION

PMN elastase from human polymorphnuclear granulocytes is a glycoprotein of 30 kDa which belongs to the group of serine proteases. Active PMN elastase is released from azurophil granula of neutrophil granulocytes after irritation or disintegration.

Indication

- Activation marker for Crohn's disease
- Chronic joint inflammation
- Bacterial infection, sepsis

3. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity
K 6841	PLATE	Microtiter plate, precoated	12 x 8 wells
K 6841	WASHBUF	ELISA wash buffer concentrate, 10 x	2 x 100 ml
K 6841	AB	Detection antibody concentrate (secondary antibody, mouse anti-PMN elastase, monoclonal), lyophilised	2 vials
K6841	CONJ	Peroxidase-labeled antibody (goat-anti-mouse-POD), ready-to-use	15 ml
K 6841	STD	Standard, lyophilised (see specification for concentration)	4 x 5 vials
K 6841	CTRL 1	Control, lyophilised (see specification for range)	4 vials
K 6841	CTRL 2	Control, lyophilised (see specification for range)	4 vials
K 6841	SUB	TMB substrate (tetramethylbenzidine), ready-to-use	15 ml
K 6841	STOP	ELISA stop solution, ready-to-use	15 ml
K 6841	SAMPLEBUF	Sample buffer, ready-to-use	100 ml

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

4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultra pure water*
- 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)

* 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 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 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 concentrate. Before dilution, the crystals have to be redissolved at room temperature or in a water bath at 37 °C. The **WASHBUF** is stable at **2–8 °C** until the expiry date stated on the label. **Wash buffer** (1:10 diluted WASHBUF) can be stored in a closed flask at **2–8 °C for 1 month**.
- The **lyophilised detection antibody concentrate (AB)** is stable at 2–8 °C until the expiry date stated on the label. Details for reconstitution and dilution are given in the specification data sheet.
- The **lyophilised standards (STD)** and **controls (CTRL)** are stable at **2–8 °C** until the expiry date stated on the label. Reconstitution details are given in the data sheet.
- 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**.

6. STORAGE AND PREPARATION OF SAMPLES

Seminal plasma

Seminal plasma should be stored at **-20 °C** and defrosted immediately before use. Centrifuge the seminal-plasma samples for **5 min** at **10 000 rpm**.

The samples should be diluted **1:10–1:20** in sample buffer (SAMPLEBUF) depending on the inflammatory status of the patient.

Serum and plasma samples

Preanalytic handling

Significant differences in the PMN elastase levels can be observed due to different sample preparation procedures, e. g. up to 10-fold higher serum levels compared to the plasma PMN elastase concentrations. The reasons are as follows:

The granulocytes are activated during the serum clotting and release elastase granulocyte-activating markers. The time between serum collecting and analysis as well as repeated freeze-thaw cycles don't cause a PMN elastase concentration shift.

On the contrary, in the case of plasma samples, varying the time between sampling and analysis or the number of freeze-thaw cycles will cause variation in the observed PMN elastase levels. Therefore, **the preanalytical conditions of plasma samples should be held constant**. This is a general requirement independent of the used test-system.

Immundiagnostik recommends the use of serum samples for PMN elastase determinations.

Fresh collected blood should be centrifuged within one hour. If not assayed on the same day, it should be stored at **-20 °C**. Lipemic or hemolytic samples should be not analysed. Samples should be mixed well before assaying. We recommend to carry out duplicate analysis on each test sample.

Serum samples should be diluted **1:500** with the sample buffer before assaying, e. g.

- **25 µl** sample + **475 µl** SAMPLEBUF, mix well = **1:20** (dilution I)
- **25 µl** dilution I + **600 µl** SAMPLEBUF, mix well = **1:25** (dilution II). This results in a **final dilution of 1:500**.

Plasma samples should be diluted **1:100** with the sample buffer before assaying,

e. g.

- **25 µl** sample + **225 µl** SAMPLEBUF, mix well = **1:10** (dilution I)
- **25 µl** dilution I + **225 µl** wash buffer, mix well = **1:10** (dilution II). This results in a **final dilution of 1:100**.

7. ASSAY PROCEDURE

Principle of the test

In a first incubation step, PMN elastase in the sample is bound to polyclonal rabbit-anti-PMN elastase antibodies (in excess), which are immobilised on the surface of the microtiter wells (PLATE). To remove all unbound substances, a washing step is carried out. In a second incubation step, a monoclonal mouse-anti-PMN elastase antibody (AB) is added. This antibody is able to detect both the free and the complexed form with the specific inhibitor (α1-proteinase inhibitor = α1-antitrypsin). The quantification of the bound PMN elastase is carried out by adding an anti-mouse peroxidase-labeled conjugate (CONJ). Finally, the PMN elastase – antigen – antibody complex is incubated with the peroxidase substrate, tetramethylbenzidine (SUB). An acidic stop solution (STOP) is then added to terminate the reaction. The color changes from blue to yellow. The intensity of the yellow color is directly proportional to the concentration of PMN elastase in the sample. A dose response curve of the absorbance unit (optical density, OD) vs. concentration is generated, using the values obtained from the calibrators. PMN elastase, present in the patient samples, is determined directly from this curve. The combination of two specific antibodies in the PMN elastase ELISA drastically reduces the possibility of false results and offers a reliable diagnostic system to the user.

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 covered at 2–8 ° C. Strips are stable until 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.	Wash each well **5 x** with **250 µl of wash buffer**. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper.
2.	Add **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.	Wash each well **5 x** with **250 µl of wash buffer**. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper.
5.	Add **100 µl of antibody solution** (diluted antibody concentrate) into each wells.
6.	Cover the strips and incubate for **1 hour** at room temperature (15–30 °C) on a **horizontal shaker.**
7.	Wash each well **5 x** with **250 µl of wash buffer**. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper.
8.	Add **100 µl conjugate** (CONJ) into each well.
9.	Cover the strips and incubate for **1 hour** at room temperature (15–30 °C) on a **horizontal shaker.**
10.	Wash each well **5 x** with **250 µl of wash buffer**. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper.
11.	Add **100 µl substrate** (SUB) into each well.
12.	Incubate for **10–20 min** at room temperature (15–30 °C) **in the dark***.
13.	Add **100 µl stop solution** (STOP) into each well, shake well.
14.	Determine absorption **immediately** with an ELISA reader at **450 nm** against 620 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.

* 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 programme used, the duplicate values should be evaluated manually.

Seminal plasma

For the calculation of the PMN elastase concentration in seminal plasma, the result has to be multiplied by the dilution factor **10 or 20** dependent on the sample dilution.

Serum

For the calculation of the PMN elastase concentration in serum, the result has to be multiplied by the **dilution factor 500**.

Plasma

For the calculation of the PMN elastase concentration in plasma, the result has to be multiplied by the **dilution factor 100**.

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 (see definition below) must be further diluted and re-assayed. Please consider this greater 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:

Analytical sensitivity × sample dilution factor to be used

10. QUALITY CONTROL

Immundiagnostik 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

PMN Elastase concentrations

in plasma of a healthy person (n = 37):	19–78 ng/ml
in serum of a healthy person (n = 52):	average = 688 ng/ml
	(186–1991 ng/ml)

We recommend each laboratory to establish its own reference range.

11. PERFORMANCE CHARACTERISTICS

Spiking Recovery

Two samples were spiked with different PMN elastase amounts and measured with the assay.

Matrix	Sample [ng/ml]	Spike [ng/ml]	PMN elastase expected [ng/ml]	PMN elastase measured [ng/ml]
Serum	0,247	0,722	0,969	0,968
	0,267	1,266	1,533	1,575

Matrix	Sample [ng/ml]	Spike [ng/ml]	PMN elastase expected [ng/ml]	PMN elastase measured [ng/ml]
Plasma	0,2	0,793	0,993	0,941
	0,21	1,285	1,495	1,467

Analytical Sensitivity

The detection limit was set as $B_0 + 1{,}645 \times SD$. The blank was measured 160 times.

Estimated detection limit = 0.011 ng/ml

This detection limit was determined in regard to the calibration curve without taking into consideration the sample dilution factor.

Dilution recovery

Two patient samples were serially diluted with ELISA wash buffer and analysed. The expected and the measured PMN elastase concentrations are displayed in the following table.

Matrix	Sample	Dilution	PMN elastase expected [ng/ml]	PMN elastase measured [ng/ml]
Serum	A	1:500	422	422
		1:1000	211	208,8
		1:2000	105,5	105,9
	B	1:500	349	349
		1:1000	174,5	170,9
		1:2000	87,25	82,1
Plasma	A	1:500	49,4	49,4
		1:1000	24,7	25,3
		1:2000	12,35	12,9
	B	1:500	19,9	19,9
		1:1000	9,95	9,7
		1:2000	4,975	5,575

Precision and reproducibility

Intra-Assay (n = 20)

Matrix	Sample	PMN elastase [ng/ml]	CV [%]
Serum	1	527,9	0,13
	2	116,4	0,5
Plasma	1	20,2	1,98
	2	20,4	2,68

Inter-Assay (n = 20)

Matrix	Sample	PMN elastase [ng/ml]	CV [%]
Serum	1	121	6,86
	2	143	5,45
Plasma	1	21	6,19
	2	27,3	11,3

Specificity

Cross reactivity with PMN elastase as well as a good correlation with PMN elastase content in mouse serum was observed.

12. PRECAUTIONS

- All reagents in the kit package are for research 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 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 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 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.
- *IDK*® is a trademark of Immundiagnostik AG.
- 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

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Used symbols:

	Temperature limitation		Catalogue Number
	In Vitro Diagnostic Medical Device		To be used with
	Manufacturer		Contains sufficient for <n> tests
	Lot number		Use by
	Attention		Consult instructions for use

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