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Manual

IDK® PMN elastase ELISA

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

Valid from 2019-02-20

REF **K 6841**

Σ 96

+2°C +8°C

IVD

CE

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1. INTENDED USE

This Immundiagnostik AG assay is an enzyme immunoassay intended for the quantitative determination of PMN elastase in serum, plasma and seminal plasma. For *in vitro* diagnostic 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 0001.C.100	WASHBUF	Wash buffer concentrate, 10 x	2 x 100 ml
K 6841	AB	Detection antibody concentrate (secondary antibody, mouse anti-PMN elastase, monoclonal), lyophilised	2 x 1 vial
K 6841	CONJ	Peroxidase-labelled antibody (goat-anti-mouse-POD), ready-to-use	1 x 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 x 1 vial
K 6841	CTRL 2	Control, lyophilised (see specification for range)	4 x 1 vial
K 6841	SUB	Substrate (tetramethylbenzidine), ready-to-use	1 x 15 ml
K 6841	STOP	Stop solution, ready-to-use	1 x 15 ml

Cat. No.	Label	Kit components	Quantity
K 6841	SAMPLEBUF	Sample dilution buffer, ready-to-use	1 x 100 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, 3000 *g*
- 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** 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**.
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- 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) 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 dilution 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 AG 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 dilution buffer (SAMPLEBUF) 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 dilution buffer (SAMPLEBUF) 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 together with the desiccant bag in the closed aluminium packaging 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.	**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/diluted 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 AB) 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 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.	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.
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 and mix 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.

* 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.

Seminal plasma

The obtained results have to be multiplied by the **dilution factor of 10 to 20 dependent on the chosen sample dilution** to get the actual concentrations.

Serum

The obtained results have to be multiplied by the **dilution factor 500** to get the actual concentrations.

Plasma

The obtained results have to be multiplied by the **dilution factor 100** 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:

Analytical sensitivity × sample dilution factor to be used

Analytical sensitivity 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

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

Accuracy – Precision

Repeatability (Intra-Assay); n = 64

The repeatability was assessed with 2 plasma samples under **constant** parameters (same operator, measurement system, day and kit lot).

Sample	Mean value [ng/ml]	CV [%]
1	542.81	6.9
2	442.08	5.0

Reproducibility (Inter-Assay); n = 46

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

Sample	Mean value [ng/ml]	CV [%]
1	131.39	6.9
2	136.60	7.2
3	123.89	8.9

Accuracy – Trueness

The trueness states the closeness of the agreement between the result of a measurement and the true value of the measurand. Therefore, PMN elastase-spikes with known concentrations were added to 2 different plasma-samples. The results below were obtained without consideration of the sample dilution factor.

Sample [ng/ml]	Spike [ng/ml]	Expected [ng/ml]	Obtained [ng/ml]	Recovery [%]
0.226	8.24	8.47	6.46	76.25
	6.63	6.86	7.49	109.23
	5.00	5.23	5.62	107.62
	3.35	3.58	3.95	110.32
	1.69	1.91	1.79	93.45
	1.01	1.24	1.09	87.71

Sample [ng/ml]	Spike [ng/ml]	Expected [ng/ml]	Obtained [ng/ml]	Recovery [%]
0.346	8.24	8.59	6.65	77.41
	6.63	6.98	5.73	82.06
	5.00	5.35	5.22	97.55
	3.35	3.70	3.65	98.68
	1.69	2.03	1.80	88.77
	1.01	1.36	1.16	85.56

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 with a serial dilution of 2 different plasma-samples.

For PMN elastase in serum, plasma and seminal plasma, the method has been demonstrated to be linear from 0.26 to 6.16 ng/ml based on the standard curve without considering possibly used sample dilution factors, showing a non-linear behaviour of less than ±20 % in this interval.

Sample	Dilution	Expected [ng/ml]	Obtained [ng/ml]	Recovery [%]
A	1:2	6.16	6.16	100.00
	1:4	3.08	3.41	110.59
	1:8	1.54	1.82	118.35
	1:16	0.77	0.91	118.35
	1:32	0.38	0.45	116.14
B	1:4	4.21	4.21	100.00
	1:8	2.10	2.13	101.21
	1:16	1.05	1.13	107.48
	1:32	0.53	0.53	100.55
	1:64	0.26	0.27	101.88

Analytical sensitivity

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

Limit of blank, LoB	0.060 ng/ml
Limit of detection, LoD	0.104 ng/ml
Limit of quantitation, LoQ	0.104 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 a range of compounds with structural similarity to PMN elastase. There was no cross-reactivity observed.

Substance tested	Concentration added	Concentration obtained [ng/ml]	Conclusion
α1-Antitrypsin	90 µg/l	< 0.060	< LoB
Albumin	800 µg/l	< 0.060	< LoB
sIgA	600 ng/ml	< 0.060	< LoB
Lysozyme	30 ng/ml	< 0.060	< LoB
Haemoglobin	1 000 µg/ml	< 0.060	< LoB
Haemoglobin-Haptoglobin-Complex	40 mU/l	< 0.060	< LoB
CRP	150 ng/ml	< 0.060	< LoB
Pancreatic Amylase	28 333 mU/l	< 0.060	< LoB
Chymotrypsin	1 000 ng/ml	< 0.060	< LoB
Myeloperoxidase	100 ng/ml	< 0.060	< LoB
Immunoglobulin E	500 ng/ml	< 0.060	< 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 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 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.
- *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

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