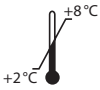
Manual

IDK® PMN-Elastase ELISA

For the determination of PMN elastase in stool

Gültig ab / Valid from 2017-11-22

REF **KR6840**



RUO CE

Immundiagnostik AG, Stubenwald-Allee 8a, 64625 Bensheim, Germany
Tel.: +49 6251 70190-0 Fax: + 49 6251 849430
e.mail: info@immundiagnostik.com www.immundiagnostik.com

distributed in the US/Canada by:

EAGLE BIOSCIENCES, INC.

20A NW Blvd, Suite 112 Nashua, NH 03063

Phone: 617-419-2019 ● FAX: 617-419-1110

www.EagleBio.com ● info@eaglebio.com

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.

Warranty Information

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

Eagle Biosciences makes no warranties, either expressed or implied, except as provided herein, including without limitation thereof, warranties as to marketability, merchantability, fitness for a particular purpose or use, or non-infringement of any intellectual property rights. In no event shall the company be liable for any indirect, incidental, or consequential damages of any nature, or losses or expenses resulting from any defective product or the use of any product. Product(s) may not be resold, modified, or altered for resale without prior written approval from Eagle Biosciences, Inc.

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

Table of Contents

1. INTENDED USE 17

2. INTRODUCTION 17

3. MATERIAL SUPPLIED 17

4. MATERIAL REQUIRED BUT NOT SUPPLIED 18

5. PREPARATION AND STORAGE OF REAGENTS 18

6. PREPARATION AND STORAGE OF SAMPLES 19

Extraction of the stool samples 19
Sample storage 20

7. ASSAY PROCEDURE 20

Principle of the test 20
Test procedure 21

8. RESULTS 22

9. LIMITATIONS 23

10. QUALITY CONTROL 23

Reference range 23

11. PERFORMANCE CHARACTERISTICS 24

Precision and reproducibility 24
Spiking Recovery 24
Dilution recovery 24
Analytical Sensitivity 25
Specificity 25

12. PRECAUTIONS 25

13. TECHNICAL HINTS 26

14. GENERAL NOTES ON THE TEST AND TEST PROCEDURE 26

15. REFERENCES 27

1. INTENDED USE

The described enzyme-linked immunosorbent assay (ELISA) is intended for the quantitative determination of PMN elastase in stool. 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. The determination of the PMN elastase in stool is used to record inflammatory reactions in which neutrophils are involved. Especially in Crohn´s disease, the inflammatory process is accompanied by an increased phagocytic activity and the biological decay of the phagocytic cells, which leads to an increased release of PMN elastase and other lysosomal enzymes.

Indications

- Activation marker for Morbus Crohn
- Chronic joint inflammation
- Bacterial infection, sepsis

3. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity
K 6840	PLATE	Microtiter plate, precoated	12 x 8 wells
K 6840	WASHBUF	ELISA wash buffer concentrate ,10 x	2 x 100 ml
K 6840	EXBUF	Extraction buffer concentrate, 2.5 x	2 x 100 ml
K 6840	AB	Detection antibody concentrate (secondary antibody, mouse anti-PMN elastase, monoclonal), lyophilised	2 vials
K6840	CONJ	Peroxidase-labeled antibody (goat-anti-mouse-POD), ready-to-use	15 ml
K 6840	STD	Standard, lyophilised (see specification for concentration)	4 x 5 vials
K 6840	CTRL 1	Control, lyophilised (see specification for range)	4 vials
K 6840	CTRL 2	Control, lyophilised (see specification for range)	4 vials

Cat. No.	Label	Kit components	Quantity
K 6840	SUB	TMB substrate (tetramethylbenzidine), ready-to-use	15 ml
K 6840	STOP	ELISA stop solution, ready-to-use	15 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
- 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. PREPARATION AND STORAGE 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**.

- **Preparation of the extraction buffer:** The **extraction buffer concentrate (EXBUF)** has to be diluted with ultra pure water **1:2.5** before use (100 ml EXBUF + 150 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 37°C in a water bath. The **EXBUF** is stable at **2–8 °C** until the expiry date stated on the label. Extraction buffer (1:2.5 diluted EXBUF) can be stored in a closed flask at **2–8 °C for 4 months**.
- 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. PREPARATION AND STORAGE OF SAMPLES

Extraction of the stool samples

Extraction buffer (1:2.5 diluted EXBUF) is used as a sample extraction buffer. We recommend the following sample preparation:

Stool Sample Application System (SAS) (Cat. No.: K 6998SAS)

Stool sample tube – Instructions for use

Please note that the dilution factor of the final stool suspension depends on the amount of stool sample used and the volume of the buffer.

SAS with 0.75 ml buffer:

Applied amount of stool:	15 mg
Buffer Volume:	0.75 ml
Dilution Factor:	1:50

Please follow the instructions for the preparation of stool samples using the SAS as follows:

a) The raw stool sample has to be thawed. For particularly heterogeneous samples we recommend a mechanical homogenisation using an applicator, inoculation loop or similar device.

b) Fill the **empty sample tube** with **0.75 ml** of prepared extraction buffer before using it with the sample. Important: Allow the extraction buffer to reach room temperature.

c) Unscrew the tube (yellow part of cap) to open. Insert the yellow dipstick into the sample. The lower part of the dipstick has notches which need to be covered completely with stool after inserting it into the sample. Place dipstick back into the tube. When putting the stick back into the tube, excess material will be stripped off, leaving 15 mg of sample to be diluted. Screw tightly to close the tube.

d) Shake the tube well until no stool sample remains in the notches. Important: Please make sure that you have a maximally homogenous suspension after shaking. Especially with more solid samples, soaking the sample in the tube with buffer for ~ 10 minutes improves the result.

e) Allow sample to stand for ~10 minutes until sediment has settled. Floating material like shells of grains can be neglected.

f) Carefully unscrew the complete cap of the tube including the blue ring plus the dipstick. Discard cap and dipstick. Make sure that the sediment will not be dispersed again.

Dilution factor: 1:50

For analysis, pipet **100 µl** of the supernatant per well.

Sample storage

Stool sample extract is stable at -20 °C for 7 days.

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, which are immobilised on the surface of the microtiter wells. To remove all unbound substances, a washing step is carried out. In a second incubation step, a monoclonal mouse-anti-PMN elastase antibody 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. Finally, the PMN elastase-antigen-antibody-complex is incubated with the peroxidase substrate, tetramethylbenzidine. An acidic stop solution is then added to terminate the reaction. The colour changes from blue to yellow. The intensity of the yellow colour 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 standards. PMN elastase, present in the patient 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.

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** of **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 of 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 of substrate** (SUB) into each well.
12.	Incubate for **10–20 min** at room temperature (15–30 °C) **in the dark***.
13.	Add **100 µl of 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 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 calibrator 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 pairs of values should be examined before the automatic evaluation of the results. If this option is not available with the programme used, the paired values should be evaluated manually.

Stool samples

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

In case **another dilution** factor has been used, multiply the obtained result with the dilution factor used to get the real concentration.

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

1 g stool is equivalent to 1 ml

PMN elastase concentrations in faeces of healthy persons (n = 76): < 62 ng/ml

We recommend each laboratory to establish its own reference concentration range.

11. PERFORMANCE CHARACTERISTICS

Precision and reproducibility

Intra-Assay (n = 20)

Sample	PMN elastase [ng/ml]	CV [%]
1	2.3	14
2	0.7	4.5

Inter-Assay (n = 20)

Sample	PMN elastase [ng/ml]	CV [%]
1	4.9	4
2	27.8	10

Spiking Recovery

Two samples were spiked with different PMN elastase calibrator amounts and measured with the *IDK*® PMN elastase ELISA.

Sample [ng/ml]	Spike [ng/ml]	expected [ng/ml]	measured [ng/ml]
1.5	3	4.5	4.9
	6	7.5	7.7
0.8	0.7	1.5	1.5
	1.7	2.5	2.7
	5	5.8	5.7

Dilution recovery

Two patient samples were diluted with ELISA wash buffer and analysed with the *IDK*® PMN elastase ELISA.

Sample	Dilution	expected [ng/ml]	measured [ng/ml]
A	1:50	22	22
	1:100	11	10.3
	1:200	5.5	5.3
	1:400	2.7	2.7
B	1:50	4.6	4.6
	1:100	2.3	2.4
	1:200	1.2	1.3
	1:400	0.6	0.9

Analytical Sensitivity

The detection limit was set as B_0 + 2 SD. The zero-standard was measured 20 times.

Sample	PMN elastase mean value [OD]	Standard deviation	Detection limit [ng/ml]
1	0.025	0.024	0.12

Specificity

No cross reactivity to other stool proteins was observed.

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 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 as wells from already opened microtiter plates are exposed to different conditions than sealed ones.
- 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.
- Quality control guidelines 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

1. Heinichen, C., Buessecker, F., Arndt, B., Schmidt-Gayk, H. & Kramer, M. D. PMN-Elastase in Faezes: Etablierung eines Lumineszenz-Immunoassays und Prüfung der diagnostischen Relevanz bei Morbus Crohn. *Clin. Lab*. **41**, 539–545 (1995).
2. Oremek, G. M. & Schneider, D. PMN-Elastase. *mta* **10**, 273–278 (1995).
3. Derhaschnig, U. et al. Recombinant human activated protein C (rhAPC; drotrecogin alfa [activated]) has minimal effect on markers of coagulation, fibrinolysis, and inflammation in acute human endotoxemia. *Blood* **102**, 2093–8 (2003).

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

distributed in the US/Canada by:

EAGLE BIOSCIENCES, INC.

20A NW Blvd, Suite 112 Nashua, NH 03063
Phone: 617-419-2019 FAX: 617-419-1110
www.EagleBio.com • info@eaglebio.com

EAGLE
BIOSCIENCES

Immundiagnostik AG

Stubenwald-Allee 8a
D-64625 Bensheim

Tel.: +49 (0) 62 51/70 19 00
Fax: +49 (0) 62 51/84 94 30

info@immundiagnostik.com
www.immundiagnostik.com