Instructions for Use

PMN-Elastase (stool) ELISA

IVD

CE

REF EIA-5699

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Please use only the valid version of the Instructions for Use provided with the kit.

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1 INTENDED USE

The assay is an enzyme immunoassay intended for the quantitative determination of PMN elastase in stool.

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.

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

Label	Kit components	Quantity
PLATE	Microtiter plate, precoated	12 x 8 wells
WASHBUF	Wash buffer concentrate, 10x	2 x 100 mL
EXBUF	Extraction buffer , ready-to-use	2 x 100 mL
AB	Detection antibody concentrate, (secondary antibody, mouse anti-PMN elastase, monoclonal), lyophilised	2 x 1 vial
CONJ	Peroxidase-labelled antibody (goat-anti-mouse-POD), ready to use	1 x 15 mL
STD	Standard, lyophilised (see specification for concentration)	4 x 5 vials
CTRL 1	Control, lyophilised (see specification for range)	4 vials
CTRL 2	Control, lyophilised (see specification for range)	4 vials
SUB	Substrate (tetramethylbenzidine), ready to use	1 x 15 mL
STOP	Stop solution, ready to use	1 x 15 mL

4 MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultrapure water*

Stool sample application system such as cat. no.: EIA-5674

- 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
- Vortex
- Standard single-use laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader (required filters see chapter 7)

* DRG 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 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 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 °C - 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 °C - 8 °C for 1 month**.

- The **lyophilised detection antibody concentrate (AB)** is stable at **2 °C - 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 °C - 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 °C - 8 °C**.

6 PREPARATION AND STORAGE OF SAMPLES

6.1 Extraction of the stool samples

Extraction buffer (EXBUF) is used as a **sample extraction buffer**.

We recommend the following sample preparation:

Stool Sample Application System (SAS) (Cat. No.: EIA-5674)

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 sample extraction 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 homogenization using an applicator, inoculation loop or similar device.

b) Fill the **empty stool sample tube** with **0.75 mL sample extraction buffer** (EXBUF) 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 sample extraction 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.

6.2 Sample storage

PMN elastase in raw stool is stable for one month at -20 °C.
Avoid repeated freezing and thawing.

Extracted stool samples are stable for 7 days at -20 °C or for 2 days at 2 °C - 8 °C.
Avoid repeated freezing and thawing as well as exposure to elevated temperatures.

7 ASSAY PROCEDURE

7.1 Principle of the test

This ELISA is designed for the quantitative determination of PMN elastase.

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-labelled 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 at 450 nm) vs. concentration is generated, using the values obtained from the standard. PMN elastase present in the patient samples, is determined directly from this curve.

7.2 Test procedure

Bring all **reagents and samples to room temperature** (15 °C - 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 °C - 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 DRG.

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 respective wells.
3. Cover the strips and incubate for **1 hour** at room temperature (15 °C - 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 detection antibody** (diluted AB) into each well.
6. Cover the strips and incubate for **1 hour** at room temperature (15 °C - 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 °C - 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 minutes**** at room temperature (15 °C - 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.

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

** 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 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 by the **dilution factor 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:

Analytical sensitivity × *sample dilution factor to be used*

Analytical sensitivity see chapter “Performance characteristics”.

10 QUALITY CONTROL

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

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. 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.

10.1 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

11.1 Accuracy - Precision

Repeatability (Intra-Assay); n = 27

The repeatability was assessed with 3 stool-samples under **constant** parameters (same operator, measurement system, day and kit lot).

Sample	Mean value [ng/mL]	CV [%]
1	62.95	8.2
2	150.06	4.2
3	57.90	9.4

Reproducibility (Inter-Assay); n = 46

The reproducibility was assessed with 3 stool-samples under varying parameters (different operators, measurement systems, days and kit lots).

Sample	Mean value [ng/mL]	CV [%]
1	158.66	12.9
2	133.66	13.9
3	186.61	14.4

11.2 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 0.269 ng/mL

Limit of quantitation, LoQ 0.309 ng/mL

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

11.3 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 high level with low level stool samples.

For PMN elastase in stool, the method has been demonstrated to be linear from 0.37 to 2.54 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	High sample	-	2.54	-
	1:1.11	2.29	2.57	111.99
	1:1.25	2.05	2.33	113.66
	1:1.43	1.80	2.10	116.78
	1:1.67	1.55	1.85	119.72
	1:2.00	1.30	1.48	113.82
	1:2.50	1.05	1.28	121.62
	1:3.33	0.80	0.99	122.96
	1:5.00	0.55	0.46	83.26
	Low sample	-	negative	-
B	High sample	-	2.29	-
	1:1.11	2.10	2.01	95.85
	1:1.25	1.90	1.73	90.65
	1:1.43	1.71	1.53	89.18
	1:1.67	1.52	1.43	94.12
	1:2.00	1.33	1.14	86.09
	1:2.50	1.14	0.99	87.38
	1:3.33	0.94	0.97	102.95
	1:5.00	0.75	0.75	99.56
	1:10.00	0.56	0.63	111.50
	Low sample	-	0.37	-

11.4 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	1000 µg/mL	< 0,060	< LoB
Haemoglobin-Haptoglobin-Complex	40 mU/L	< 0,060	< LoB
CRP	150 ng/mL	< 0,060	< LoB
Pancreatic Amylase	28333 mU/L	< 0,060	< LoB
Chymotrypsin	1000 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 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 up immediately with copious quantities of water. Do not breath vapor 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 analyzed with each run.
- Reagents should not be used beyond the expiration date stated on kit label.
- Substrate solution should remain colorless 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.
- Quality control guidelines 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. DRG can therefore not be held responsible for any damage resulting from incorrect use.

15 REFERENCES / LITERATURE

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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).

SYMBOLS USED

Symbol	English	Deutsch	Italiano	Español	Français
CE	European Conformity	CE-Konformitäts-kennzeichnung	Conformità europea	Conformidad europea	Conformité normes européennes
	Consult instructions for use *	Gebrauchsanweisung beachten *	Consultare le istruzioni per l'uso	Consulte las instrucciones de uso	Consulter les instructions d'utilisation
IVD	*In vitro* diagnostic medical device *	*In-vitro*-Diagnostikum *	Dispositivo medico-diagnostico in vitro	Producto sanitario para diagnóstico In vitro	Dispositif médical de diagnostic in vitro
REF	Catalogue number *	Artikelnummer *	Numero di Catalogo	Nûmero de catálogo	Référence de catalogue
LOT	Batch code *	Chargencode *	Codice del lotto	Codigo de lote	Numéro de lot
	Contains sufficient for <n> tests *	Ausreichend für <n> Prüfungen *	Contenuto sufficiente per "n" saggi	Contenido suficiente para <n> ensayos	Contenu suffisant pour "n" tests
	Temperature limit *	Temperaturbegrenzung *	Temperatura di conservazione	Temperatura de conservacion	Température de conservation
	Use-by date *	Verwendbar bis *	Utilizzare prima del	Establa hasta	Utiliser jusque
	Manufacturer *	Hersteller *	Fabbricante	Fabricante	Fabricant
	Caution *	Achtung *			
RUO	For research use only	Nur für Forschungszwecke	Solo a scopo di ricerca	Sólo para uso en investigación	Seulement dans le cadre de recherches
Distributed by	Distributed by	Vertreiber	Distributore	Distribuidor	Distributeur
Content	Content	Inhalt	Contenuto	Contenido	Contenu
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantità	Volumen/Número	Volume/Quantité