BioVendor Group®

ENG

Instructions for Use:

HUMAN PMN ELASTASE ELISA

Catalogue number:

RM191021100

Example Version

For research use only!

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1. INTRODUCTION 4
2. PRINCIPLE 5
3. WARNINGS AND PRECAUTIONS 5
4. REAGENTS 6
5. SPECIMEN COLLECTION AND PREPARATION 8
6. ASSAY PROCEDURE 9
7. EXPECTED NORMAL VALUES 12
8. QUALITY CONTROL 12
9. PERFORMANCE CHARACTERISTICS 13
10. LIMITATIONS OF PROCEDURE 17
11. LEGAL ASPECTS 18
12. REFERENCES 19
13. SHORT INSTRUCTION 20
14. EXPLANATION OF SYMBOLS 21

Example Version

HISTORY OF CHANGES

Previous version	Current version
ENG.008.A	ENG.009.A
Chapter 4.2.; 4.4.1 Polypropylene tubes added	
Chapter 6.1	
in double determinations	in duplicates
Chapter 6.4 changed optical density values	
Chapter 7 changed values in the table	
Chapter 9.1	
The lowest analytical detectable level of PMN Elastase that can be distinguished from the Zero Calibrator is 0.1 ng/ml at the 2SD confidence limit.	The lowest analytical detectable level of PMN Elastase that can be distinguished from the Zero Calibrator is 0.03 ng/ml at the 2SD confidence limit without taking any dilution factor into account
Chapter 9.3	
Measurement range (from analytical sensitivity to highest calibrator): 0.1 to 10 ng/ml.	Measurement range (from analytical sensitivity to highest calibrator): 0.03 to 10 ng/ml
9.4.1	
The intra-assay variation was determined by 20 replicate measurements of EDTA / citrate plasma and seminal plasma within one run. The within-assay variability is shown below:	The intra-assay variation was determined by 20 replicate measurements of EDTA / citrate plasma and seminal plasma within one run with PMN Elastase ELISA. The intra-assay variability is shown below:
The values in the tables have been changed	
9.4.2	
The inter-assay (between-run) variation was determined by duplicate measurements of samples in at least ten different tests	The inter-assay (between-run) variation was determined by duplicate measurements of samples in at least ten different runs with PMN Elastase ELISA.
The values in the tables have been changed	
9.5	
Each sample (non-spiked and spiked) was assayed and analyte concentrations of the samples were calculated from the standard curve. The percentage recoveries were determined by comparing expected and measured values of the samples.	Each sample (non-spiked and spiked) was assayed with PMN Elastase ELISA and analyte concentrations of the samples were calculated from the standard curve. The percentage recoveries were determined by comparing expected and observed values of the samples.
The values in the tables have been changed	
9.6	
Plasma (EDTA, citrate) and seminal plasma containing different amounts of analyte were serially diluted with Dilution Buffer and assayed	Plasma (EDTA, citrate) and seminal plasma containing different amounts of analyte were serially diluted with Dilution Buffer and assayed with PMN Elastase
The values in the tables have been changed	

Example Version

10.3	
High Dose Hook Effect is not detected in the range between 0-620 ng/ml, based on Calibrator range (0-10 ng/ml).	High Dose Hook Effect is not detected in the range between 0-900 ng/ml, based on Calibrator range (0-10 ng/ml).

1. INTRODUCTION

1.1 Intended use

The BioVendor PMN Elastase ELISA is an enzyme immunoassay for the quantitative determination of the complex of PMN Elastase and the α1-proteinase inhibitor (α1-PI) in human EDTA or citrated plasma and seminal plasma. The assay is intended for research use only. Manual processing is validated. The usage of laboratory automates is the user's sole responsibility. The kit is intended for single use only.

1.2 Description of the analyte

The human organism reacts with an inflammatory response to attacks of invading pathogens (microorganisms and viruses) or damaged tissue (after accidents or surgery). Polymorphonuclear (PMN) granulocytes play an important role as primary defense cells in this inflammatory reaction. Different bloodstream mediators (cytokines, leukotrienes, complement factors, bacterial endotoxins, clotting, and fibrinolysis factors) attract and stimulate these cells to phagocytose and destroy not naturally occurring agents.
PMN granulocytes use proteinases to digest these agents and tissue debris. One of these proteinases is PMN Elastase, which is localized in the azurophilic granules of the PMN granulocytes. During phagocytosis of foreign substances, these enzymes are also partially excreted into the extracellular surrounding, where the activity of PMN Elastase is regulated by inhibitors (esp. the α_1-proteinase inhibitor, α_1PI). An overwhelming release of PMN Elastase, however, can exceed the inhibitory potential of the $\alpha_1$proteinase inhibitor. Thus, enzymatically active PMN Elastase, together with simultaneously produced oxidants (O_2-radicals, H_2O_2, OH-radicals, etc.), can cause local tissue injury.
Due to the bloodstream and lymphatic system, α_1-PI is delivered subsequently and eventually able to form a complex with all excreted PMN Elastase. Therefore, the concentration of the PMN Elastase/ α_1PI complex correlates with the released PMN Elastase and can be used as a measure for the activity of granulocytes during an inflammatory response.
Primarily, determinations of PMN Elastase find its application as an adjunct in the diagnostics and observation of the course of trauma, shock and sepsis (1-3). Further indications are the areas of hemodialysis, infections by obstetrics, joint diseases, intestinal affection and pancreatitis (8-13). The determination of PMN Elastase in seminal plasma can be used as an adjunct in the diagnostics of male adnex affections (5-7).

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2. PRINCIPLE

The test kit is a solid phase enzyme-linked immunosorbent assay (ELISA) in the microplate format, designed for the quantitative measurement of the complex of human PMN Elastase and α_1-proteinase inhibitor (α_1-PI) in blood plasma and seminal plasma.
The microplate is coated with a polyclonal antibody directed against human PMN Elastase (antigen). Calibrators, controls and patient samples are pipetted into the antibody-coated microplate. During a 60 minutes incubation, antigens present in the sample bind to the antibodies fixed on the inner surface of the wells. Non-reactive sample components are removed by a washing step.
Afterwards, a second polyclonal antibody directed against α_1-PI, which is labeled with horseradish peroxidase (Conjugate Solution), is added. During a 60 minutes incubation, the PMN Elastase/ α_1-PI complex bound to the first antibody is specifically recognized by the enzyme-labeled second antibodies, and a sandwich complex is formed. Excess of conjugate solution is washed out.
A chromogenic substrate, TMB (3,3',5,5'-Tetramethylbenzidine), is added. During a 30 minutes incubation, the substrate is converted to a colored endproduct (blue) by the fixed enzyme. Enzyme reaction is stopped by dispensing of hydrochloric acid as stop solution (change from blue to yellow). The color intensity is directly proportional to the concentration of PMN Elastase present in the sample. The optical density of the color solution is measured with a microtiter plate reader at 450 nm.

Example Version

3. WARNINGS AND PRECAUTIONS

1. This kit is for research use only.
2. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
3. All human source material used in the preparation of the reagents has been tested and found negative for antibodies to HIV 1&2, HbsAg, and HCV. No test method however can offer complete assurance that HIV, HBV, HCV or other infectious agents are absent. Therefore, the reagents must be handled in the same manner as potentially infectious material.
4. The microtiter plate contains break apart strips. Unused wells must be stored at 2-8°C in the sealed foil pouch and used in the frame provided.
5. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
6. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing substrate solution that had previously been used for conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
7. Mix the contents of the microtiter plate wells thoroughly to ensure good test results. Do not reuse microwells.
8. Do not let wells dry during assay; add reagents immediately after completing the washing steps.
9. Allow the reagents to reach room temperature (18-25°C) before starting the test. Temperature will affect the absorbance readings of the assay.
10. Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
11. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
12. Wear disposable protective gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.

13. Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
14. Do not use reagents beyond expiry date as shown on the kit labels.
15. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiter plate readers.
16. Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may be slightly different.
17. Avoid contact with Stop Solution. It may cause skin irritation and burns.
18. Some reagents contain Proclin 300, CMIT and/or MIT as preservatives. In case of contact with eyes or skin, flush immediately with water.
19. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
20. For information on hazardous substances included in the kit please refer to Safety Data Sheets. Safety Data Sheets for this product are available upon request directly from BioVendor (www.biovendor.com)
21. If product information, including labeling, is incorrect or inaccurate, please contact the kit manufacturer or supplier.

4. REAGENTS

4.1 Reagents supplied

Kit Components	State	Quantity
Antibody Coated Microtiter Strips	ready to use	96 wells
Conjugate Solution	ready to use	16 ml
Master Standard	lyophilized	1 vial
Quality Control HIGH	lyophilized	1 vial
Quality Control LOW	lyophilized	1 vial
Dilution Buffer	ready to use	50 ml
Wash Solution Conc. (10x)	concentrated	50 ml
Substrate Solution	ready to use	22 ml
Stop Solution	ready to use	7 ml

Example Version

4.2 Materials required but not supplied

- Microtiter plate photometer with optical filter for 450 nm
- Polypropylene tubes
- Vortex mixer
- Microtiter plate shaker operating at 900 rpm
- Deionized water
- Manual or automatic equipment for microtiter plate washing
- Absorbent paper
- Timing device
- Calibrated variable micropipettes and multichannel pipettes with disposable pipette tips
- Semilogarithmic paper or software for data processing

4.3 Storage conditions

When stored at 2-8°C unopened reagents will be stable until expiration date. Do not use reagents beyond this date. Opened reagents must be stored at 2-8°C. After first opening, the reagents are stable for 30 days if performed and stored properly. Keep reagents away from heat and direct sunlight.
The Wash Solution is stable for 12 weeks at room temperature (18-25°C) after dilution.
Store Calibrators and Controls at 2-8°C for 7 days or at ≤-20 °C (in aliquots) for 30 days after reconstitution. Microtiter wells must be stored at 2-8°C. Once the foil bag has been opened, care should be taken to close it tightly again. Protect TMB Substrate Solution from light.

4.4 Preparation of reagents

4.4.1 PMN Elastase Master Standard:

Reconstitute lyophilized Master Standard with 2 ml Dilution Buffer 30 minutes before use (final concentration of 10 ng/ml). Make a dilution series with Dilution Buffer to get calibrators with 5, 2.5, 1.25, 0.63, 0.31, and 0.16 ng/ml, respectively.
Label six polypropylene tubes: G (5 ng/ml), F (2.5 ng/ml), E (1.25 ng/ml), D (0.63 ng/ml), C (0.31 ng/ml), and B (0.16 ng/ml). The reconstituted PMN Elastase Master Standard will serve as the highest calibrator H (10 ng/ml). Use the Dilution Buffer as the zero calibrator (A).
Pipet 0.5 ml of the Dilution Buffer into all tubes (B-G). Pipet 0.5 ml of the reconstituted PMN Elastase Master Standard into tube G (5 ng/ml) and mix thoroughly. Transfer 0.5 ml from tube G (5 ng/ml) to tube F (2.5 ng/ml) and mix thoroughly. Repeat this process successively to complete the 2-fold dilution series.

4.4.2 PMN Elastase Controls:

Reconstitute with 1 ml Dilution Buffer 30 minutes before use. The PMN Elastase Controls must not be diluted.

4.4.3 Wash Solution:

Dilute 50 ml of 10x concentrated Wash Solution with 450 ml deionized water to a final volume of 500 ml. The diluted Wash Solution is stable for at least 12 weeks at room temperature (18-25°C). Precipitates may form when stored at 2-8°C, which should dissolve again by swirling at room temperature (18-25°C). The Wash Solution should only be used when the precipitates have completely dissolved.

Example Version

4.5 Disposal of the kit

The disposal of the kit must be made according to the national regulations. Special information for this product is given in the Safety Data Sheet.

4.6 Damaged test kit

In case of any severe damage of the test kit or components, BioVendor has to be informed in writing within one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.

5. SPECIMEN COLLECTION AND PREPARATION

For determination of PMN Elastase EDTA or citrated plasma as well as seminal plasma are the preferred sample matrixes. It is important that the preanalytics are constant.

EDTA or citrated plasma: The usual precautions for venipuncture should be observed (14). It is important to preserve the chemical integrity of a blood specimen from the moment it is collected until it is assayed. Collect blood by venipuncture into vacutainers and separate plasma immediately from cells by centrifugation. Use plasma samples at the same day. For longer storage (up to 12 months), samples should be stored frozen at ≤-20°C. To avoid repeated thawing and freezing the samples must be aliquoted. Mix the samples before use.
Do not use hemolytic, icteric or lipemic specimens. Furthermore, we recommend special caution when using gel collection systems, as an influence on the measurement results cannot be excluded in case of improper handling. Samples containing sodium azide should not be used in the assay.

Seminal plasma. Separate the sperm by centrifugation. Take the supernatant for the assay. Freeze the seminal plasma at ≤ -20°C for longer storage up to 12 months. To avoid repeated thawing and freezing the samples must be aliquoted. Mix the samples before use.

Serum is not suitable, because during clotting PMN Elastase can be released *in vitro*.

Dilution of samples:
Dilute EDTA or citrated plasma samples 1:100 with **Dilution Buffer** immediately before assay. Therefore, 10 µl of sample must be diluted with 990 µl of Dilution Buffer in a polypropylene tube. After mixing, use 100 µl/well of this 1:100 diluted sample in the assay. Do not store the diluted sample for a longer time, it has to be used freshly.
Please note: The dilution factor 100 has to be taken into account for the calculation of the results.

Dilute **seminal plasma samples 1:200** with **Dilution Buffer** immediately before assay. Therefore, 5 µl of sample must be diluted with 995 µl of **Dilution Buffer** in a polypropylene tube. After mixing, use 100 µl/well of this 1:200 diluted sample in the assay. Do not store the diluted sample for a longer time, it has to be used freshly.
Please note: The dilution factor 200 has to be taken into account for the calculation of the results.

Patient samples expected to contain higher PMN Elastase concentrations than the highest calibrator (10 ng/ml) should be further diluted with **Dilution Buffer** prior to assaying. The additional dilution step has to be taken into account for the calculation of the results.

Example Version

6. ASSAY PROCEDURE

6.1 General remarks

- Do not interchange components of different lots.
- All components of these test kits supplied as concentrate should be diluted to their final concentration at least 30 minutes prior to use. Mix well, but prevent foam formation.
- All reagents and specimens must be allowed to come to room temperature (18-25°C) before use. All reagents must be mixed without foaming.
- Once the test has been started, all steps should be completed without interruption.
- Use new disposal plastic pipette tips for each standard, control or sample in order to avoid crosscontamination.
- Optical density is a function of the incubation time and temperature. Before starting the assay, it is recommended that all samples are diluted, reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- As a general rule the enzymatic reaction is linearly proportional to time and temperature.
- Respect the incubation times as stated in this instruction for use.
- Calibrators, controls and samples should at least be assayed in duplicates.
- Microtiter plate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or a multistepper, respectively, or an automatic microtiter plate washing system. Do not allow the wells to dry between incubations. Do not scratch coated wells during rinsing and aspiration. Rinse and fill all reagents with care. While rinsing, check that all wells are filled precisely with Wash Solution, and that there are no residues in the wells.
- A calibrator curve must be established for every test run.

Example version

6.2 Assay procedure

1. **Preparation of Calibrators (see chapter 4.4)**:
   Label six tubes: G (5 ng/ml), F (2.5 ng/ml), E (1.25 ng/ml), D (0.63 ng/ml), C (0.31 ng/ml), and B (0.16 ng/ml). Pipet **0.5 ml** of the **Dilution Buffer** into all tubes (G-B). Pipet 0.5 ml of the **reconstituted PMN Elastase Master Standard** into tube G (5 ng/ml) and mix thoroughly. Transfer 0.5 ml from tube G (5 ng/ml) to tube F (2.5 ng/ml) and mix thoroughly. Repeat this process successively to complete the 2-fold dilution series. The reconstituted PMN Elastase Master Standard will serve as the highest calibrator H (10 ng/ml). Use the PMN Elastase Dilution Buffer as the zero calibrator A (0 ng/ml).
2. Dilute **EDTA or citrate plasma samples 1:100** and **seminal plasma samples 1:200**, respectively, with **Dilution Buffer** in a polypropylene tube prior to assaying as described in chapter 5. Reconstituted Controls must not be diluted.
3. Prepare a sufficient number of microplate wells to accomodate Calibrators, Controls and prediluted patient samples in duplicates.

	1	2	3	4	5	6	7	8	9	10	11	12
a	A	E	C1	P..								
b	A	E	C1	P..								
c	B	F	C2									
d	B	F	C2									
e	C	G	P1									
f	C	G	P1									
g	D	H	P2									
h	D	H	P2									

Example Version

4. For determination of PMN Elastase pipet **100 µl** of **Calibrators**, **Controls and prediluted patient samples** with new disposable tips into the wells according to the template.
5. Incubate for **60 minutes** at room temperature (18-25°C) on a plate mixer (900 rpm).
6. Decant the content of the wells and wash **4 times** with **300 µl Wash Solution**. Remove as much Wash Solution as possible by beating the microplate carefully on absorbent paper.
7. Pipet **150 µl** of **Conjugate Solution** into each well.
8. Incubate for **60 minutes** at room temperature (18-25°C) on a plate mixer (900 rpm).
9. Again, decant the content of all wells and wash **4 times** with **300 µl Wash Solution**. Remove as much wash solution as possible by beating the microplate carefully on absorbent paper.
10. Dispense **200 µl** of **TMB Substrate Solution** into each well.
11. Incubate for **30 minutes** at room temperature (18-25°C) in the dark without shaking.
12. Add **50 µl** of **Stop Solution** to each well and mix carefully.
13. Determine the optical density of each well at 450 nm and read the wells within 15 minutes.

6.3 Calculation of results

1. Calculate the average optical density values for each set of Calibrators, Controls and samples.
2. The obtained optical density of the Calibrators (y-axis, linear) are plotted against their corresponding concentrations (x-axis, logarithmic) either on semilogarithmic paper or using an automated method.
3. Using the mean optical density value for each sample, determine the corresponding concentration from the calibration curve.
4. Automated method: The results in the package insert have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred calculation method. Other data reduction functions may give slightly different results.
5. The PMN Elastase concentration of the sample can be calculated by multiplication with the respective dilution factor (see chapter 5).
6. Samples with concentrations higher than the highest calibrator (10 ng/ml) have to be further diluted. For the calculation of the concentration, this dilution factor also has to be taken into account.

6.4 Example of typical calibrator curve

The figure below shows typical results for BioVendor PMN Elastase ELISA. These data are intended for illustration only and must not be used to calculate results from another run.

Calibrator	Optical Density (450 nm)
Calibrator A 0 ng/ml	0.102
Calibrator B 0.16 ng/ml	0.229
Calibrator C 0.31 ng/ml	0.369
Calibrator D 0.63 ng/ml	0.655
Calibrator E 1.25 ng/ml	1.143
Calibrator F 2.5 ng/ml	1.784
Calibrator G 5.0 ng/ml	2.513
Calibrator H 10.0 ng/ml	2.986

Example Version

7. EXPECTED NORMAL VALUES

The following values are observed with plasma and seminal plasma of apparently healthy adults with the BioVendor PMN Elastase ELISA:

		ng/ml			
	n	**Range**	**Median**	**2.5 percentile**	**97.5 percentile**
citrate plasma	45	23.2-81.6	37.4	25.2	69.5
EDTA plasma	45	19.5-55.3	32.4	22.4	48.7
seminal plasma	40	1.9-624.5	34.0	5.4	362.3

n.d. not detectable

The results alone should not be the only reason for any diagnostic or therapeutical consequences. They have to be correlated to other clinical observations and diagnostic tests.
Positive results should be verified concerning the entire clinical status of the patient. Also, every decision for therapy should be taken individually. It is recommended that each laboratory establishes its own normal and pathological ranges of PMN Elastase. The reference ranges should be regarded as guidelines only.

Example Version

8. QUALITY CONTROL

Good laboratory practice requires that controls are run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance. It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day-to-day validity of results. Use controls at both normal and pathological levels.
The controls and the corresponding results are stated in the QC certificate included in the kit. The values and ranges stated on the QC certificate always refer to the current kit lot and should be used for direct comparison of the results. It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.
Employ appropriate statistical methods for analyzing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials, patient results should be considered invalid. In this case, please check the following technical areas: Pipetting and timing devices, microtiter plate reader, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods. After checking the above mentioned items without finding any error contact your distributor or BioVendor directly.

9. PERFORMANCE CHARACTERISTICS

9.1 Analytical sensitivity

The lowest analytical detectable level of PMN Elastase that can be distinguished from the Zero Calibrator is 0.03 ng/ml at the 2SD confidence limit without taking any dilution factor into account.

9.2 Specificity

The BioVendor PMN Elastase ELISA specifically detects human PMN elastase/α_1-PI complex.

9.3 Measurement range

Measurement range (from analytical sensitivity to highest calibrator): 0.03 to 10 ng/ml.

9.4 Reproducibility

9.4.1 Intra-Assay

The intra-assay variation was determined by 20 replicate measurements of EDTA / citrate plasma and seminal plasma within one run with PMN Elastase ELISA. The intra-assay variability is shown below:

EDTA plasma	n	Mean (ng/ml)	CV (%)
1	20	287.5	9.9
2	20	26.3	6.2

citrate plasma	n	Mean (ng/ml)	CV (%)
1	20	51.6	9.1
2	20	107.2	3.4

seminal plasma	n	Mean (ng/ml)	CV (%)
1	20	231.7	3.0
2	20	546.1	2.8
3	20	169.0	2.0
4	20	61.7	2.7

Example Version

9.4.2 Inter-Assay

The inter-assay (between-run) variation was determined by duplicate measurements of samples in at least ten different runs with PMN Elastase ELISA.

EDTA plasma	n	Mean (ng/ml)	CV (%)
1	10	273.8	10.6
2	10	27.4	7.2

citrate plasma	n	Mean (ng/ml)	CV (%)
1	10	46.6	8.1
2	10	104.2	4.1

seminal plasma	n	Mean (ng/ml)	CV (%)
1	10	242.3	11.2
2	10	511.6	9.8
3	10	153.9	6.4
4	10	58.1	8.6

9.5 Recovery

Recovery was determined by adding increasing amounts of the analyte to different samples containing different amounts of endogenous analyte. Each sample (non-spiked and spiked) was assayed with PMN Elastase ELISA and analyte concentrations of the samples were calculated from the standard curve. The percentage recoveries were determined by comparing expected and observed values of the samples.

EDTA plasma	Spiking (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery (%)
1	-	190.5	-	-
	+ 50	242.4	240.5	101
	+ 150	365.5	340.5	107
	+ 300	576.3	490.5	117
2	-	113.1	-	-
	+ 50	174.3	163.1	107
	+ 150	283.4	263.1	108
	+ 300	475.7	413.1	115

citrate plasma	Spiking (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery (%)
1	-	28.1	-	-
	+ 50	77.7	78.1	99
	+ 150	168.4	178.1	95
	+ 300	338.8	328.1	103
2	-	52.4	-	-
	+ 50	109.4	102.4	107
	+ 150	210.3	202.4	104
	+ 300	375.4	352.4	107

Example Version

seminal plasma	Spiking (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery (%)
1	-	41.9	-	-
	+ 50	94.6	91.9	103
	+ 150	182.8	191.9	95
	+ 300	322.3	341.9	94
2	-	438.3	-	-
	+ 50	612.4	488.3	125
	+ 150	672.0	588.3	114
	+ 300	760.4	738.3	103
3	-	246.9	-	-
	+ 50	305.7	296.9	103
	+ 150	390.2	396.9	98
	+ 300	528.8	546.9	97

9.6 Linearity

Plasma (EDTA, citrate) and seminal plasma containing different amounts of analyte were serially diluted with Dilution Buffer and assayed with PMN Elastase. The percentage linearity was calculated by comparing the expected and measured values.

Example Version

EDTA plasma	Dilution	Observed (ng/ml)	Expected (ng/ml)	Linearity (%)
1	-	308.6	-	-
	1 : 2	145.2	154.3	94
	1 : 4	69.1	77.1	90
	1 : 8	37.36	38.6	97
2	-	172.9	-	-
	1 : 2	88.5	86.5	102
	1 : 4	45.2	43.2	105
	1 : 8	23.8	21.6	110

citrate plasma	Dilution	Observed (ng/ml)	Expected (ng/ml)	Linearity (%)
1	-	107.9	-	-
	1 : 2	57.5	54.0	107
	1 : 4	31.8	27.0	118
	1 : 8	17.2	13.5	128
2	-	58.8	-	-
	1 : 2	32.0	29.4	109
	1 : 4	14.4	14.7	98
	1 : 8	8.6	7.4	117

seminal plasma	Dilution	Observed (ng/ml)	Expected (ng/ml)	Linearity (%)
1	-	478.4	-	-
	1 : 2	261.4	239.2	109
	1 : 4	129.9	119.6	109
	1 : 8	68.6	59.8	115
2	-	412.5	-	-
	1 : 2	229.3	206.3	111
	1 : 4	116.5	103.1	113
	1 : 8	63.2	51.6	123
3	-	624.5	-	-
	1 : 2	335.4	312.3	107
	1 : 4	165.6	156.1	106
	1 : 8	90.5	78.1	116

Example Version

10. LIMITATIONS OF PROCEDURE

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the package insert instruction and with adherence to good laboratory practice. Any improper handling of samples or modification of this test might influence the results.

10.1 Interfering substances

- Hemoglobin (up to 30 mg/dl), bilirubin (up to 40 mg/dl), and lipids (up to 30 mg/ml) show no significant influence on the assay results. However, we recommend not to use any hemolytic, icteric or lipemic specimens to avoid any interferences.
- Samples containing sodium azide should not be used in the assay.
- The result of any immunological test system may be affected by heterophilic antibodies, anti-species antibodies or rheumatoid factors present in human samples (15-17). For example, the presence of heterophilic antibodies in patients who are regularly exposed to animals or animal products may interfere with immunological tests. Therefore, interference with *this in vitro* immunoassay cannot be excluded. If unplausible results are suspected, they should be considered invalid and verified by further testing. For diagnostic purposes, results should always be considered only in conjunction with the patient's clinical picture and further diagnostic tests.

10.2 Drug Interferences

Until today no substances (drugs) are known to us, which have an influence to the measurement of PMN Elastase in samples. Any medication should be taken into account when assessing the results.

10.3 High-Dose-Hook Effect

High Dose Hook Effect is not detected in the range between 0-900 ng/ml, based on Calibrator range (0-10 ng/ml).

Example Version

11. LEGAL ASPECTS

11.1 Reliability of results

The test must be performed exactly as per the manufacturer's instructions for use. Moreover, the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include a sufficient number of controls within the test procedure for validating the accuracy and precision of the test.
The test results are valid only if all controls meet the specified ranges and if all other test parameters are also within the given assay specifications. In case of any doubt or concern, please contact BioVendor.

11.2 Therapeutic consquences

Therapeutic consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated under point 11.1. Any laboratory result is only a part of the total clinical picture of a patient.
Only in cases where the laboratory results are in acceptable agreement with the overall clinical picture of the patient therapeutic consequences should be derived. The test result itself should never be the sole determinant for deriving any therapeutic consequences.

11.3 Liability

Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.
Claims submitted due to customer misinterpretation of laboratory results subject to point 11.2. are also invalid. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

Example version

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Example Version

13. SHORT INSTRUCTION

MP Well / Steps	ng/ml / Solution	A	B	C	D	E	F	G	H	Control 1/2	Sample
		0	**0.16**	**0.31**	**0.63**	**1.25**	**2.5**	**5**	**10**		
Steps	**Solution**										
Pipet	Master Standard	100	100	100	100	100	100	100	100	-	-
Pipet	Control	-	-	-	-	-	-	-	-	100	
Pipet	Prediluted sample	-	-	-	-	-	-	-	-	-	100
Incubate for **60 min** at RT (18-25°C) on a plate mixer (900 rpm)											
Decant Wash **4x with 300µl** of Wash solution											
Pipet	Conjugate Solution	150	150	150	150	150	150	150	150	150	150
Incubate for **60 min** at RT (18-25°C) on a plate mixer (900 rpm)											
Decant Wash **4x with 300µl** of Wash solution											
Pipet	Substrate Solution	200	200	200	200	200	200	200	200	200	200
Incubate for **30 min** at RT (18-25°C) without shaking **in the dark**											
Pipet	Stop Solution	50	50	50	50	50	50	50	50	50	50
Read at λ = **450nm**											

Example Version

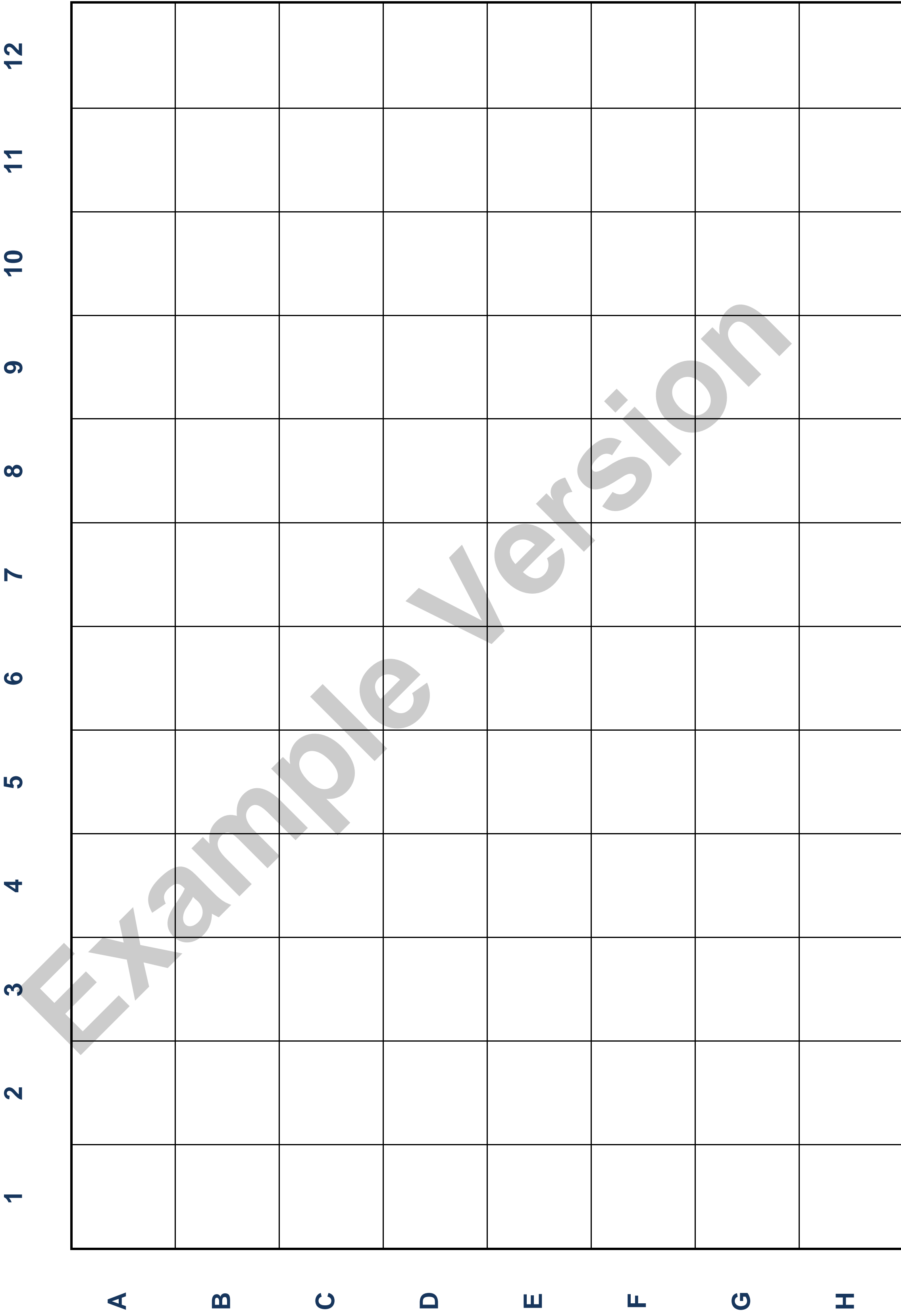
All sample sizes given in µl.

For a detailed description of the procedure see also chapter 6.2.

14. EXPLANATION OF SYMBOLS

Symbol	Explanation
REF	Catalogue number
LOT	Batch code
	Caution
	Use by date
2 °C – 8 °C	Temperature limit
	Manufacturer
www.biovendor.com	Read electronic instructions for use - eIFU
Σ 96	The content is sufficient for 96 tests
	Biological risks

Example Version



Example Version

Example Version

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