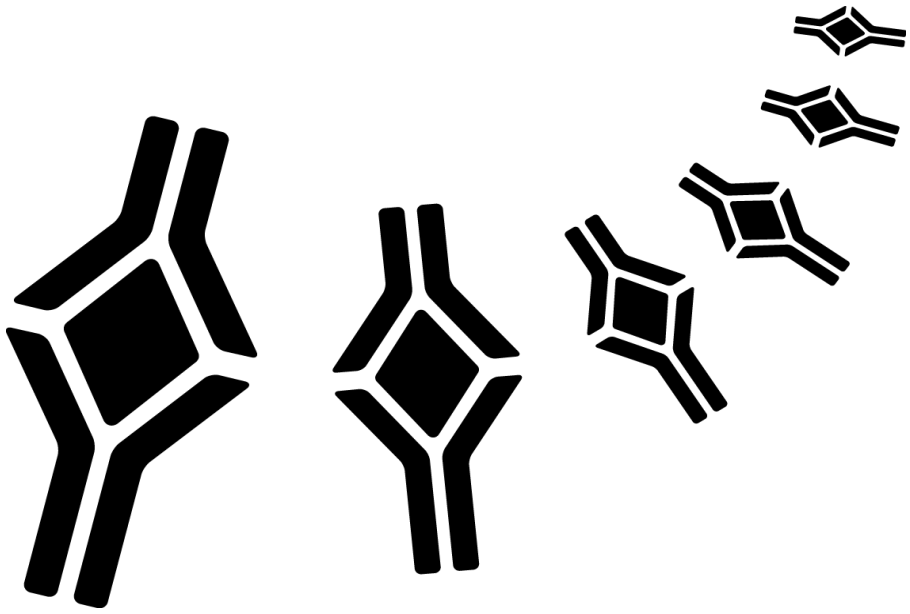
BioVendor
Research
and Diagnostic Products



HUMAN PMN ELASTASE ELISA

Product Data Sheet

Cat. No.: RM191021100

European Union: IVD CE

Rest of the world:
For research use only!

CONTENTS

» **This kit is manufactured by:**
BioVendor – Laboratorní medicína a.s.

» **Use only the current version of Product Data Sheet enclosed with the kit!**

1. INTENDED USE

The RM191021100 Human PMN Elastase ELISA is a sandwich enzyme immunoassay for the quantitative measurement of the complex of human PMN elastase and α1-proteinase inhibitor (α1-PI).

» Features

- **European Union: for in vitro diagnostic use.**
  Rest of the world: for research use only!
- The total assay time is less than 3 hours.
- The kit measures PMN elastase in plasma (EDTA and citrate), exudate, bronchoalveolar lavage fluid, cerebrospinal fluid and seminal plasma.
- Assay format is 96 wells.
- Components of the kit are provided ready to use, concentrated or lyophilized.

2. STORAGE, EXPIRATION

Store the complete kit at 2-8°C. Under these conditions, the kit is stable until the expiration date (see label on the box).

For stability of opened reagents see Chapter 9.

3. INTRODUCTION

The human organism reacts with an inflammatory response to attacks of invading pathogens (micro-organisms and viruses) or damaged tissue (after accidents or surgery). Polymorphonuclear (PMN) granulocytes play an important role as primary defence cells in this inflammatory reaction. Different bloodstream mediators (cytokines, leukotrienes, complement factors, bacterial endotoxins, clotting and fibrinolysis factors) attract and stimulate these cells to phagocytize 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 localised in the azurophilic granules of the polymorphonuclear 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 α1-proteinase inhibitor, α1-PI). An overwhelming release of PMN elastase, however, can exceed the inhibitory potential of the α1-proteinase inhibitor. Thus, enzymatically active PMN elastase, together with simultaneously produced oxidants (O_2-radicals, H_2O_2, OH-radicals), can cause local tissue injury.
Due to the bloodstream and lymphatic system, however, α1-PI is delivered subsequently and eventually able to form a complex with all excreted elastase. Therefore, the concentration of the PMN elastase/ α1-PI 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 in observation of the course of trauma, shock and sepsis. Further indications are the areas of hemodialysis, infections by obstetrics, joint diseases, effusions of sport injuries, intestinal affection, pancreatitis, cystic fibrosis and male adnex affections.

4. TEST PRINCIPLE

In the BioVendor Human PMN Elastase ELISA, Standards, Quality Controls and samples are incubated in microplate wells pre-coated with polyclonal antibody. After 60 minutes incubation and washing, polyclonal antibody, conjugated with horseradish peroxidase (HRP) is added to the wells and incubated for 60 minutes with captured PMN elastase/α1-PI complex. Following another washing step, the remaining HRP conjugate is allowed to react with the substrate solution (TMB). The reaction is stopped by addition of acidic solution and absorbance of the resulting yellow product is measured. The absorbance is proportional to the concentration of PMN elastase. A standard curve is constructed by plotting absorbance values against concentrations of Standards, and concentrations of unknown samples are determined using this standard curve.

5. PRECAUTIONS

- **For professional use only.**
- Wear gloves and laboratory coats when handling immunodiagnostic materials.
- Do not drink, eat or smoke in the areas where immunodiagnostic materials are being handled.
- This kit contains components of human origin. These materials were found non-reactive for HBsAg, HCV antibody and for HIV 1/2 antigen and antibody. However, these materials should be handled as potentially infectious, as no test can guarantee the complete absence of infectious agents.
- Avoid contact with the acidic Stop Solution and Substrate Solution, which contains hydrogen peroxide and tetramethylbenzidine (TMB). Wear gloves and eye and clothing protection when handling these reagents. Stop and/or Substrate Solutions may cause skin/eyes irritation. In case of contact with the Stop Solution and the Substrate Solution wash skin/eyes thoroughly with water and seek medical attention, when necessary.
- The materials must not be pipetted by mouth.

6. TECHNICAL HINTS

- Reagents with different lot numbers should not be mixed.
- Use thoroughly clean glassware.
- Use deionized (distilled) water, stored in clean containers.
- Avoid any contamination among samples and reagents. For this purpose, disposable tips should be used for each sample and reagent.
- Substrate Solution should remain colourless until added to the plate. Keep Substrate Solution protected from light.
- Stop Solution should remain colourless until added to the plate. The colour developed in the wells will turn from blue to yellow immediately after the addition of the Stop Solution. Wells that are green in colour indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.
- Dispose of consumable materials and unused contents in accordance with applicable national regulatory requirements.

7. REAGENT 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
Product Data Sheet + Certificate of Analysis		1 pc

8. MATERIAL REQUIRED BUT NOT SUPPLIED

- Deionized (distilled) water
- Test tubes for diluting samples
- Glassware (graduated cylinder and bottle) for Wash Solution (Dilution Buffer)
- Precision pipettes to deliver 10-1000 µl with disposable tips
- Multichannel pipette to deliver 20-200 µl with disposable tips
- Absorbent material (e.g. paper towels) for blotting the microtitrate plate after washing
- Vortex mixer
- Orbital microplate shaker capable of approximately 350 – 400 rpm
- Microplate washer (optional). [Manual washing is possible but not preferable.]
- Microplate reader with 450 ± 10 nm filter, preferably with reference wavelength 630 nm (alternatively another one from the interval 550-650 nm)
- Software package facilitating data generation and analysis (optional)

9. PREPARATION OF REAGENTS

» **All reagents need to be brought to room temperature prior to use.**

» **Always prepare only the appropriate quantity of reagents for your test.**

» **Do not use components after the expiration date marked on their label.**

- **Assay reagents supplied ready to use:**

Antibody Coated Microtiter Strips
Stability and storage:
Return the unused strips to the provided aluminium zip-sealed bag with desicant and seal carefully. Remaining Microtiter Strips are stable 3 months stored at 2-8°C and protected from the moisture.

Conjugate solution
Dilution Buffer
Substrate Solution
Stop Solution
Stability and storage:
Opened reagents are stable 3 months when stored at 2-8°C.

- **Assay reagents supplied concentrated or lyophilized:**

Master Standard
Reconstitute the lyophilized Master Standard with **2 ml** of Dilution Buffer just prior to the assay. Let it dissolve at least 30 minutes with occasionally gently shaking (not to foam). The resulting concentration in the stock solution is **1000 ng/ml**.

Prepare set of standards using Dilution Buffer as follows:

Volume of Standard	Dilution Buffer	Concentration
Stock	-	1000 ng/ml
500 µl of stock	500 µl	500 ng/ml
500 µl of 500 ng/ml	500 µl	250 ng/ml
500 µl of 250 ng/ml	500 µl	125 ng/ml
500 µl of 125 ng/ml	500 µl	62.5 ng/ml
500 µl of 62.5 ng/ml	500 µl	31.3 ng/ml
500 µl of 31.3 ng/ml	500 µl	15.6 ng/ml

Prepared Standards are ready to use, do not dilute them.

Stability and storage:

Set of standards (1000 – 15.6 ng/ml) should be aliquoted and frozen at –20°C for 1 month. Avoid repeated freeze/thaw cycles.

Do not store the diluted samples.

Quality Controls HIGH, LOW

Reconstitute each Quality Control (HIGH and LOW) with **1 ml** of Dilution Buffer just prior to the assay. Let it dissolve at least 30 minutes with occasionally gently shaking (not to foam).

Reconstituted Quality Controls are ready to use, do not dilute them.

Stability and storage:

The reconstituted Quality Controls must be used immediately or aliquoted and frozen at -20°C for 1 month. Avoid repeated freeze/thaw cycles.

Do not store the diluted samples.

Wash Solution Conc. (10x)

Dilute Wash Solution Concentrate (10x) ten-fold in distilled water to prepare a 1x working solution. Example: 75 ml of Wash Solution Concentrate (10x) + 675 ml of distilled water for use of all 96-wells.

Stability and storage:

The diluted Wash Solution is stable 6 month when stored at 2-8°C. Opened Wash Solution Concentrate (10x) is stable 3 months when stored at 2-8°C.

10. PREPARATION OF SAMPLES

For determination of PMN elastase EDTA or citrated plasma are the preferred sample matrixies. Exudate, bronchoalveolar lavage fluid, cerebrospinal fluid and seminal plasma can be used.
Serum is not suitable, because during clotting PMN elastase can be released *in vitro*. Culture supernatants are as well not suitable; the reason is that the assay detects only the PMN elastase/ α1-PI complex and α1-PI is normally not present in culture medium.

The patients need not be fasting, and no special preparations are necessary. Collect blood by venipuncture into vacutainers and separate plasma from the cells by centrifugation.
In case of measurement of PMN elastase in seminal plasma, separate the seminal plasma by centrifugaion (5 min.) and take the supernatant.

Samples should be assayed immediately after collection or should be stored at -20°C. Mix thoroughly thawed samples just prior to the assay and avoid repeated freeze/thaw cycles, which may cause erroneous results. Avoid using hemolyzed or lipemic samples.

Dilute samples 100x with Dilution Buffer just prior to the assay, e.g. 10 µl l of sample + 990 µl of Dilution Buffer for duplicates. **Mix well** (not to foam). Vortex is recommended.

Stability and storage:
Samples should be stored at -20°C, or preferably at -70°C for long-term storage. Avoid repeated freeze/ thaw cycles.
Do not store the diluted samples.

Note: It is recommended to use a precision pipette and a careful technique to perform the dilution in order to get precise results.

11. ASSAY PROCEDURE

1. Pipet **100 µl** of diluted Standards, Quality Controls, Dilution Buffer (=Blank) and samples, preferably in duplicates, into the appropriate wells.
2. Incubate the plate at room temperature (18 – 28 °C) for **1 hour**, shaking at **350 – 400 rpm** on an orbital microplate shaker.
3. Wash the wells **4-times** with Wash Solution (0.30 ml per well). After final wash, invert and tap the plate strongly against paper towel.
4. Add **150 µl** of Conjugate Solution into each well.
5. Incubate the plate at room temperature (18 – 28 °C) for **1 hour**, shaking at **350 – 400 rpm** on an orbital microplate shaker.
6. Wash the wells **4-times** with Wash Solution (0.30 ml per well). After final wash, invert and tap the plate strongly against paper towel.
7. Add **200 µl** of Substrate Solution into each well. Avoid exposing the microtiter plate to direct sunlight. Covering the plate with e.g. aluminium foil is recommended.
8. Incubate the plate for **20 minutes** at room temperature (18 – 28 °C). Do not shake the plate during the incubation.
9. Stop the colour development by adding **50 µl** of Stop Solution.
10. Determine the absorbance of each well using a microplate reader set to 450 nm, preferably with the reference wavelength set to 630 nm (acceptable range: 550 – 650 nm). Subtract readings at 630 nm (550 - 650 nm) from the readings at 450 nm. The absorbance should be read within 5 minutes following step 9.

Note: If some samples and standard/s have absorbances above the upper limit of your microplate reader, perform a second reading at 405 nm. A new standard curve, constructed using the values measured at 405 nm, is used to determine PMN elastase concentration of off-scale standards and samples. The readings at 405 nm should not replace the readings for samples that were "in range" at 450 nm.

Note 2: Manual washing: Aspirate wells and pipet 0.35 ml Wash Solution into each well. Aspirate wells and repeat twice. After final wash, invert and tap the plate strongly against paper towel. Make certain that Wash Solution has been removed entirely.

	strip 1+2	strip 3+4	strip 5+6	strip 7+8	strip 9+10	strip 11+12
A	**Standard 1000**	**QC High**	Sample 7	Sample 15	Sample 23	Sample 31
B	**Standard 500**	**QC Low**	Sample 8	Sample 16	Sample 24	Sample 32
C	**Standard 250**	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
D	**Standard 125**	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
E	**Standard 62.5**	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
F	**Standard 31.3**	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
G	**Standard 15.6**	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
H	**Blank**	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38

Figure 1: Example of a work sheet.

12. CALCULATIONS

Most microplate readers perform automatic calculations of analyte concentration. The standard curve is constructed by plotting the mean absorbance (Y) of Standards against the known concentration (X) of Standards in logarithmic scale, using the four-parameter algorithm. Results are reported as concentration of PMN elastase ng/ml in samples.

Alternatively, the logit log function can be used to linearize the standard curve, i.e. logit of the mean absorbance (Y) is plotted against log of the known concentration (X) of Standards.

The concentration read from the standard curve is the real concentration of PMN elastase in the sample, no dilution factor is applied.

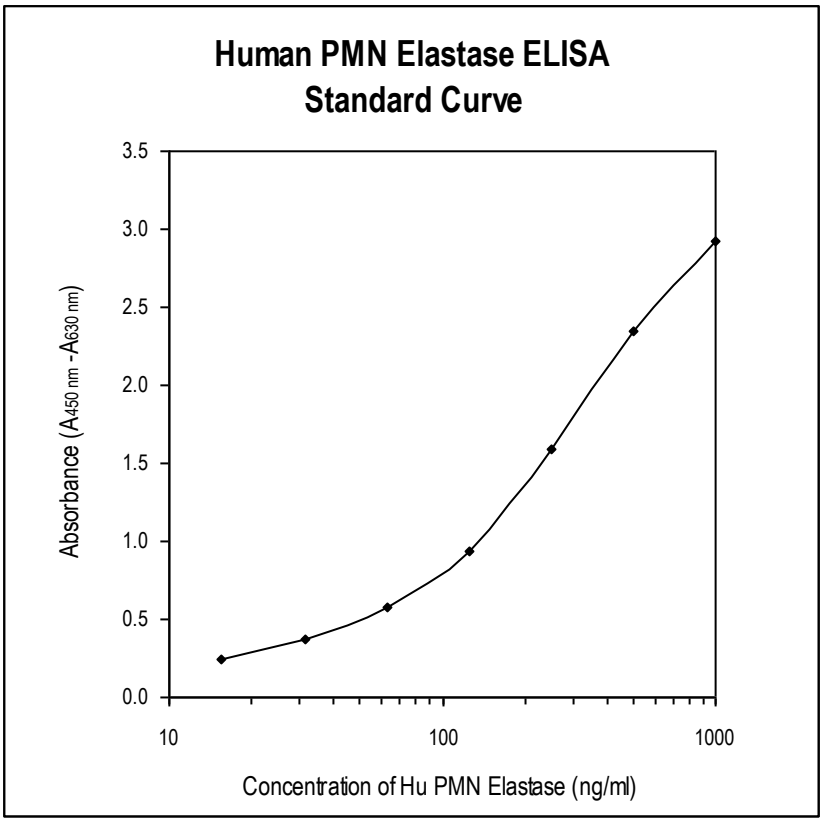


Figure 2: Typical Standard Curve for Human PMN Elastase ELISA.

13. PERFORMANCE CHARACTERISTICS

Typical analytical data of BioVendor Human PMN Elastase ELISA are presented in this chapter.

- **Sensitivity**

Limit of Detection (LOD) (defined as concentration of analyte giving absorbance higher than mean absorbance of blank* plus three standard deviations of the absorbance of blank: A_{blank} + $3xSD_{blank}$) is calculated from the real PMN elastase values in wells and is 0.2 ng/ml.
*Dilution Buffer is pipetted into blank wells.

- **Limit of Assay**

Results exceeding PMN elastase level of 1000 ng/ml should be repeated with more diluted samples. Dilution factor higher than 1:100 must be taken into account and the results must be multiplied by dilution factor/100. For example, when the samples are diluted 1:200, the results must be multiplied by 200/100, it means multiplication by 2.

- **Specificity**

The BioVendor PMN elastase test kit is specific for human PMN elastase only, respectively the PMN elastase/α1-PI complex.

Sera of several mammalian species were measured in the assay. See results below.
For details please contact us at info@biovendor.com.

Mammalian serum sample	*Observed crossreactivity*
Bovine	no
Cat	no
Dog	no
Goat	no
Hamster	no
Horse	no
Monkey	yes
Mouse	no
Pig	no
Rabbit	no
Rat	no
Sheep	no

- **Precision**

Intra-assay (Within-Run) (n=10)

Sample	*Mean (ng/ml)*	*CV (%)*
1	80	5.2
2	241	4.7
3	358	4.6

Inter-assay (Run-to-Run) (n=5)

Sample	*Mean (ng/ml)*	*CV (%)*
1	128	5.7
2	216	6.4
3	346	4.4
4	681	5.7

- **Spiking Recovery**

Plasma samples were spiked with different amounts of human PMN elastase and assayed.

Sample	***O**bserved (ng/ml)*	***E**xpected (ng/ml)*	*Recovery **O/E** (%)*
1	23.2	-	-
	72.4	69.4	104
	59.3	54.1	109
	49.6	47.2	101
2	30.6	-	-
	73.4	76.7	96
	59.3	61.4	97
	56.8	54.4	104
3	61.7	-	-
	118.0	107.8	109
	100.8	92.5	109
	94.8	85.6	110

- **Linearity**

Plasma samples were serially diluted with Dilution Buffer and assayed.

Sample	*Dilution*	***O**bserved (ng/ml)*	***E**xpected (ng/ml)*	*Recovery **O/E** (%)*
1	-	250.1	-	-
	2x	145.2	125.1	116
	4x	75.3	62.5	120
	8x	37.1	31.3	119
2	-	465.5	-	-
	2x	209.2	232.8	90
	4x	111.1	116.4	95
	8x	58.7	58.2	101

- **Effect of Bilirubin and Hemolysis**

To simulate moderate and severe icterus, four samples were spiked with 100 and 200 milligrams of bilirubin per liter. All samples were assayed, both spiked and unspiked, with the following results (ng/ml). The results show that severe icterus (bilirubin up to 200 mg/L) has no clinically significant effect on the BioVendor PMN elastase procedure.

Samples with hemolysis normally show no effect on the BioVendor PMN elastase procedure. In single cases hemolysis can lead to an increase due to the decay of granulocytes *in vitro*.

Sample No.	*Unspiked (ng/ml)*	*+ 100 mg/l Bilirubin*	*+ 200 mg/l Bilirubin*
1	100	106	104
2	249	245	261
3	572	575	534
4	903	964	910

- **Normal values**

In a normal range study with plasma samples from healthy blood donors (n = 57) the following ranges have been established with the BioVendor Human PMN Elastase ELISA.

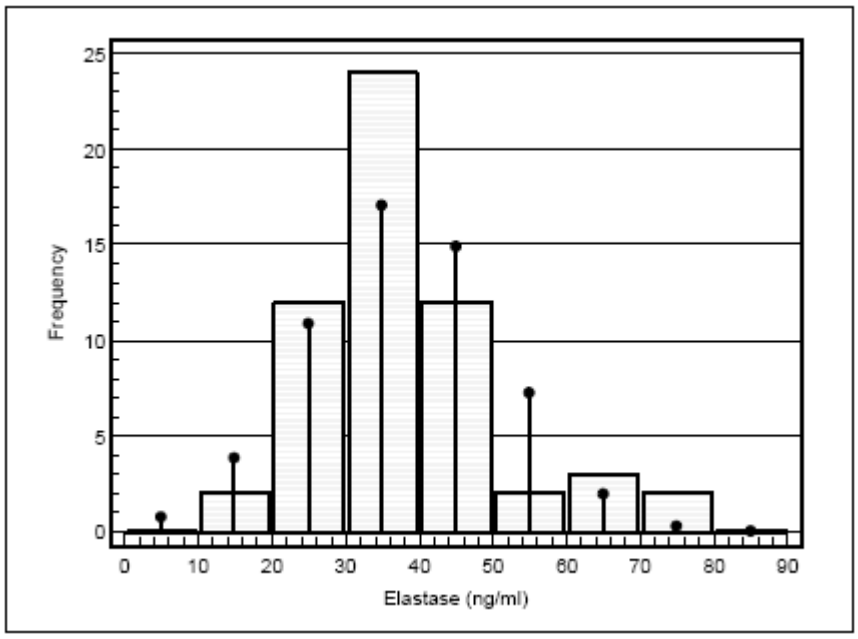


Figure 3: Frequency distribution of PMN elastase in citrated plasma of healthy blood donors (median = 35 ng/ml, 95 % percentile = 64.9 ng/ml).

- **Interpretation of the seminal plasma results**

Interpretation	*Concentration (ng/ml)*
no inflammation	0 - 250
moderate inflammation	> 250 – 1 000
massive inflammation	> 1 000

Positive results should be verified concerning the entire clinical status of the patient. Also every decision for therapy should be taken individually. The data quoted in these instructions should be used for guidance only. Each laboratory should establish its own normal and pathological ranges for PMN elastase levels with the assay. Each laboratory should establish a quality control program to monitor the quality of the assay.

14. TROUBLESHOOTING AND FAQS

›› Weak signal in all wells

Possible explanations:

- Omission of a reagent or a step
- Improper preparation or storage of a reagent
- Assay performed before reagents were allowed to come to room temperature
- Improper wavelength when reading absorbance

›› High signal and background in all wells

Possible explanations:

- Improper or inadequate washing
- Overdeveloping; incubation time with Substrate Solution should be decreased before addition of Stop Solution
- Incubation temperature over 30°C

›› High coefficient of variation (CV)

Possible explanation:

- Improper or inadequate washing
- Improper mixing Standards, Quality Controls or samples

15. REFERENCES

›› References to Human PMN Elastase ELISA:

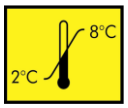
- Jochum M., Machleidt, W., Neuhof, H., and Fritz, H. Proteinases. In: Schlag G, Redl H (eds), Pathophysiology of Shock, Sepsis, and Organ Failure. Springer-Verlag Berlin Heidelberg, 1993: 46-60.
- Jochum M., Machleidt, W., and Fritz, H. Proteolytic Enzyme Systems. In: Schlag G, Redl H (eds), Pathophysiology of Shock, Sepsis, and Organ Failure. Springer-Verlag Berlin Heidelberg, 1993: 531-548.
- Elastase In: Thomas, L. (Hrsg.) Labor und Diagnose Die Medizinische Verlagsgesellschaft, 4. Auflage, 1992: 795 – 801
- Elastase, Elastase-α1-Proteinase Inhibitor Complex In: Friedman, R.B., Young, D.S (Eds.) Effects of Disease on Clinical Laboratory Tests AACC Press, Washington, 3rd Edition, 1997: 3-161
- Reinhardt, A., Haidl, G., and Schill, W-B. Granulocyte elastase indicates silent male genital tract inflammation and appropriate antiinflammatory treatment. Andrologia 29, 1996: 187 – 192

›› References to this product:

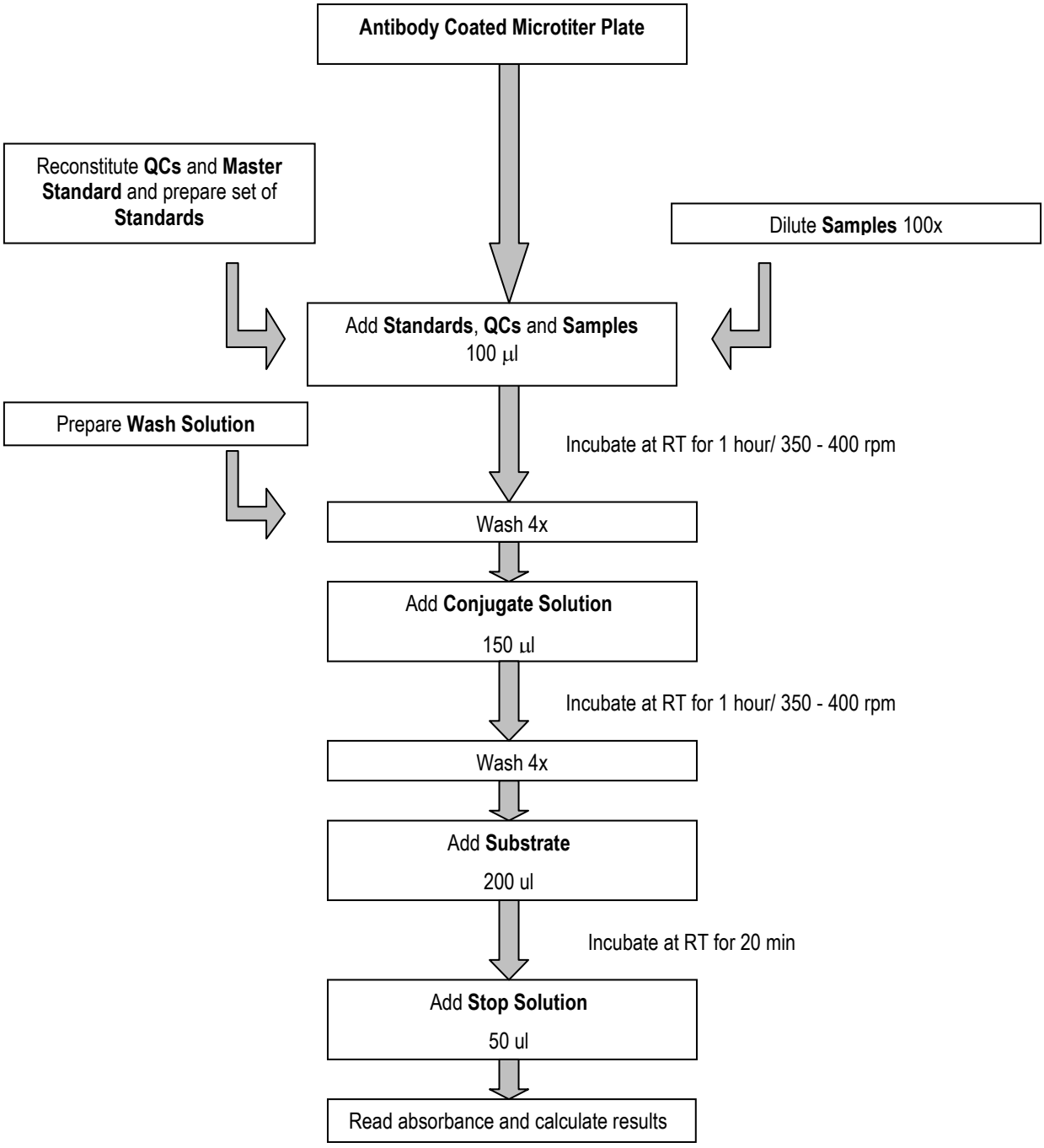
- Park CJ, Clark SG, Lichtensteiger CA, Jamison RD, Johnson AJ. *Accelerated wound closure of pressure ulcers in aged mice by chitosan scaffolds with and without bFGF.* Acta Biomater. 2009 Jul;5 (6):1926-36
- Naskalski JW, Kapusta M, Fedak D, Dumnicka P, Kusnierz-Cabala B, Kuzniewski M, Sulowicz W. *Effect of Hemodialysis on Acid Leukocyte-Type Ribonuclease, Alkaline Ribonuclease and Polymorphonuclear Elastase Serum Levels in Patients with End-Stage Renal Disease.* Nephron Clin Pract. 2009 Jun 16;112 (4):c248-c254
- Laing SJ, Jackson AR, Walters R, Lloyd-Jones E, Whitham M, Maassen N, Walsh NP. *Human blood neutrophil responses to prolonged exercise with and without a thermal clamp.* J Appl Physiol. 2008 Jan;104 (1):20-6
- Huang Y, Xie K, Zhang J, Dang Y, Qiong Z. *Prospective clinical and experimental studies on the cardioprotective effect of ulinastatin following severe burns.* Burns. 2008 Aug;34 (5):674-80
- Allan EK, Holyoake TL, Jorgensen HG . *Human neutrophil elastase is not a target for therapy in chronic myeloid leukaemia.* Leukemia. 2006 Nov;20(11):2054-5
- Davison G, Gleeson M . *The effect of 2 weeks vitamin C supplementation on immunoendocrine responses to 2.5 h cycling exercise in man.* Eur J Appl Physiol. 2006 Jul;97(4):454-61

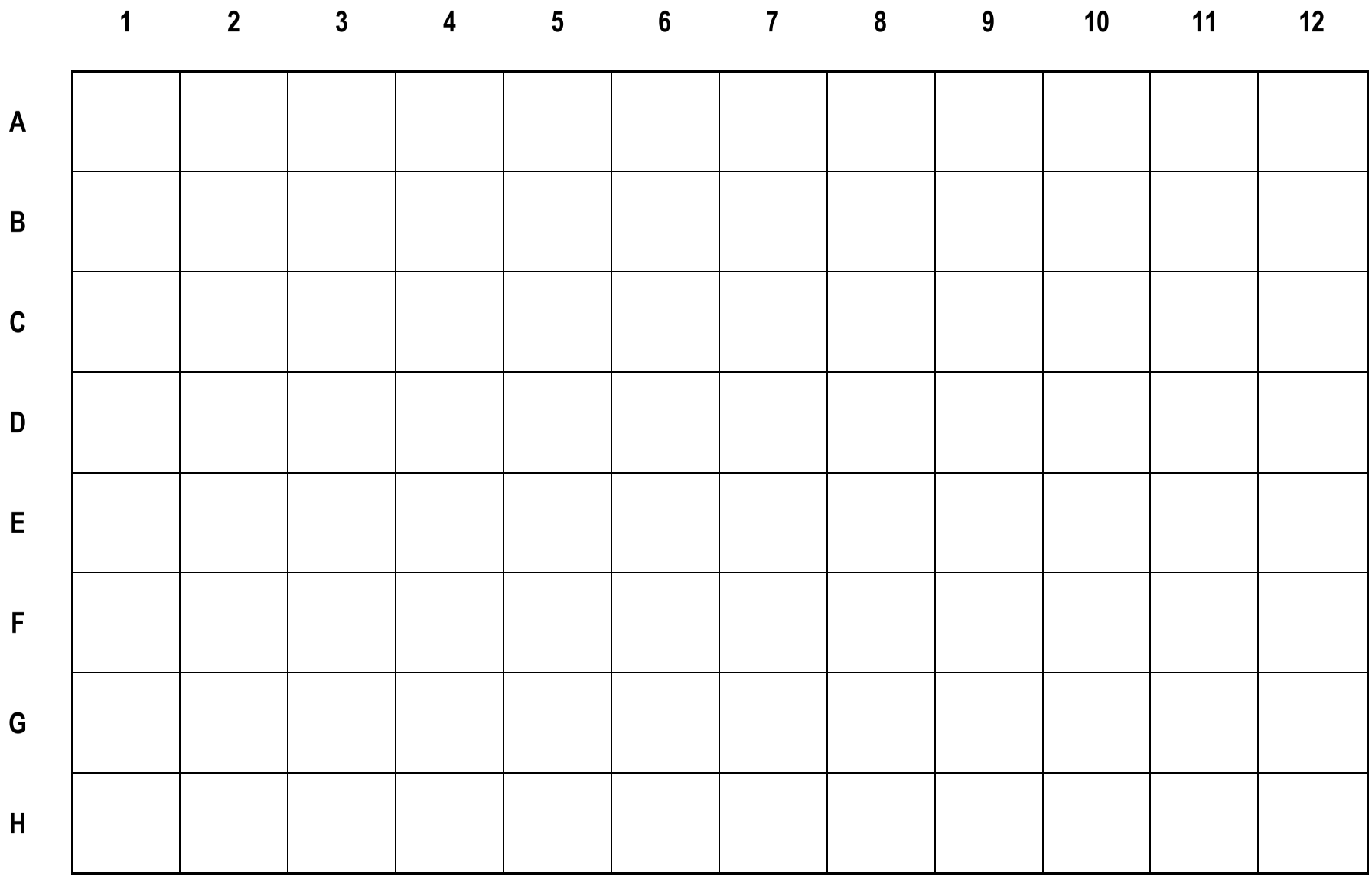
›› For more references on this product see our WebPages at www.biovendor.com

16. EXPLANATION OF SYMBOLS

REF	Catalogue number
Cont.	Content
LOT	Lot number
	See instructions for use
	Biological hazard
	Expiry date
	Storage conditions
	Identification of packaging materials
IVD CE	In vitro diagnostic medical device

Assay Procedure Summary

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NOTES

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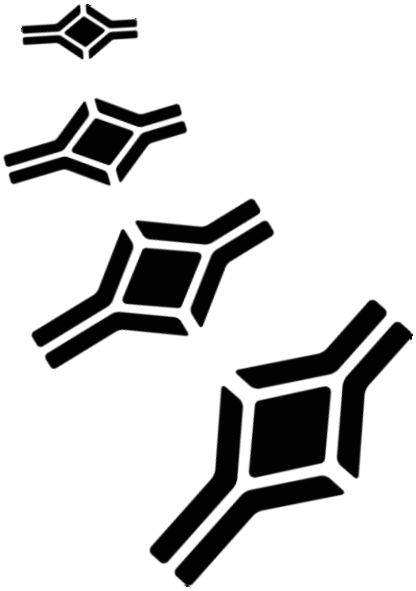

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