Human PMN Elastase ELISA Kit

Enzyme Immunoassay for the quantification of human PMN Elastase in EDTA or citrate plasma, exudate, bronchoalveolar lavage fluid, cerebrospinal fluid and seminal plasma

Catalog number: ARG80507

distributed in the US/Canada by:
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For research use only. Not for use in diagnostic procedures.

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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 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 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 localized 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 (O2-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, and effusions of sport injuries, intestinal affection, pancreatitis, cystic fibrosis and male adnex affections.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for PMN Elastase has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any PMN Elastase present is bound by the immobilized antibody. After washing away any unbound substances, a HRP-conjugated antibody specific for PMN Elastase is added to each well and incubate. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of PMN Elastase bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450 nm ±2 nm.The concentration of PMN Elastase in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	1 plate	4°C. Unused strips should be sealed tightly in the air-tight pouch.
Standards	1 vial	4°C, Lyophilized
Control Serum	2 vials	4°C, Lyophilized
Enzyme-conjugated Antibody	1 vial	4°C, ready for use
Sample Diluent	50 ml	4°C, ready for use
10X Wash buffer	50 ml	4°C
TMB substrate	22 ml	4°C (Protect from light)
STOP solution	7 ml	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Briefly spin down the antibody conjugate concentrate and HRP-Streptavidin concentrate before use.

- If crystals are observed in the 20X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Plasma - Collect plasma using EDTA or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Seminal plasma- Separate the seminal plasma by centrifugation (5 min).

Take the supernatant and freeze the seminal plasma at –20°C for longer storage.

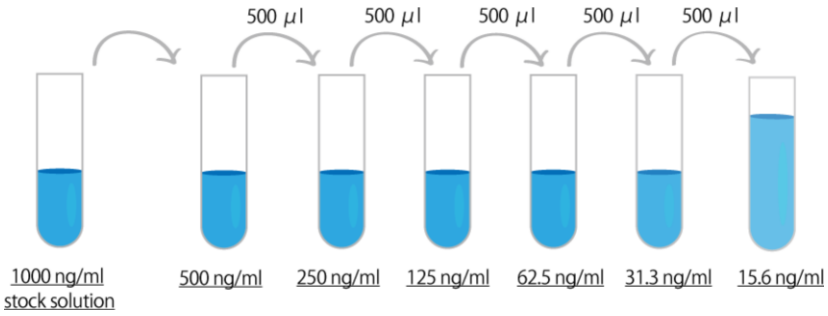
Note: Serum is not suitable for the kit, because during clotting PMN elastase can be released in vitro. Culture supernatants are as well not suitable for this kit; the reason is that the assay detects only the PMN elastase/α1-PI complex and α1 PI is normally not present in culture medium.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 10X Wash buffer into distilled water to yield 1X Wash buffer.
- Controls: Control serum/buffer matrix are lyophilized contain human serum and should be reconstituted in 1 ml Sample Diluent each. Reconstituted controls are ready to use and need not to be further

diluted.

- **Samples**: All samples should be diluted 1:100-fold with Sample Diluent. Therefore 10 µL of sample may be diluted with 990 µL of Sample diluent. Samples expected to contain higher PMN elastase concentrations than the highest standard (1000 ng/mL) should be diluted in the Sample Diluent further before assaying. The additional dilution step has to be taken into account for the calculation of the results.
- **Standards**: Reconstitute the standard with 2 ml Sample Diluent to yield a stock concentration of 1000 ng/ml (Reconstituted standard are ready to use and need not to be further diluted). Make sure the standard is dissolved completely before making serial dilutions. The Sample Diluent serves as zero standard (0 pg/ml), and the rest of the standard serial dilution can be diluted into assay buffer as according to the suggested concentration below: 1000 ng/ml, 500 ng/ml, 250 ng/ml, 125 ng/ml, 62.5 ng/ml, 31.3 ng/ml, and 15.6 ng/ml. Use the Sample Diluent as zero calibrator (0 ng/ml).



ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 100 µl of standards, controls and samples in duplicate into wells.
3. Cover the wells and incubate for 1 hour at RT.
4. Aspirate each well and wash, repeating the process 3 times for a total 4 washes. Wash by filling each well with 1× Wash Buffer (350 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
5. Add 150 µl of Enzyme-conjugated Antibody to each well. Cover wells and incubate for 1 hour at RT.
6. Aspirate each well and wash as step 4.
7. Add 200 µl of TMB Reagent to each well. Incubate for 20 minutes at room temperature in dark.
8. Add 50 µl of Stop Solution to each well.
9. Read the OD with a microplate reader at 450 nm immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

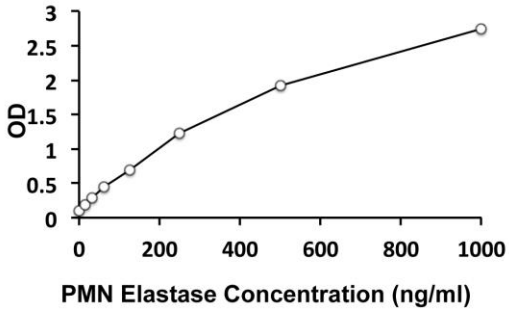
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Specificity

The PMN Elastase test is specific human PMN Elastase only, respectively the PMN Elastase/α_1-PI complex.

Sensitivity

The minimum detectable dose (MDD) of PMN Elastase ranged from 0-1000 ng/ml. The mean MDD was 0.2 ng/ml.

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 4.8 % and inter-assay precision was 5.55%.

Recovery

96-110%

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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.

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