ab119553 -
PMN Elastase
Human ELISA Kit

Instructions for Use

For the quantitative measurement of Human PMN Elastase concentrations in Cell culture supernatants, plasma, exudate, bronchoalveolar lavage fluid, cerebrospinal fluid and seminal plasma.

This product is for research use only and is not intended for diagnostic use.
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INTRODUCTION

1. BACKGROUND

Abcam’s PMN Elastase Human in vitro ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for accurate quantitative measurement of Human PMN Elastase concentrations in Cell culture supernatants, plasma, exudate, bronchoalveolar lavage fluid, cerebrospinal fluid and seminal plasma.

Human PMN Elastase specific antibodies have been precoated onto 96-well plates. Standards and test samples are added to the wells and then incubated at room temperature. After washing, a HRP-conjugated anti-human alpha1-PI detection antibody is added then incubated at room temperature. Following washing Streptavidin-HRP conjugate is added to each well, incubated at room temperature then again washed. TMB is added and then catalyzed by HRP to produce a blue color product that changes into yellow after adding acidic stop solution. The density of yellow coloration is directly proportional to the amount of Human PMN Elastase captured on the plate.

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 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, alpha1-PI). An overwhelming release of PMN Elastase,
however, can exceed the inhibitory potential of the alpha1-proteinase inhibitor. Thus, enzymatically active PMN Elastase, together with simultaneously produced oxidants (O$_2$-radicals, H$_2$O$_2$, OH-radicals), can cause local tissue injury.

Due to the bloodstream and lymphatic system, however, α$_1$-PI is delivered subsequently and eventually able to form a complex with all excreted elastase. Therefore, the concentration of the PMN Elastase/α$_1$-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.
2. **ASSAY SUMMARY**

**Primary capture antibody**

Equilibrate all reagents to room temperature. Prepare all the reagents, samples, and standards as instructed.

**Sample**

Add standard or sample to each well used. Incubate the plate.

**HRP conjugated antibody**

Wash and add prepared HRP-conjugated anti-human alpha1-PI antibody. Incubate at room temperature.

**Substrate**

Wash and add TMB Substrate to each well. Add Stop Solution to each well. Read immediately.
3. PRECAUTIONS
Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY
Upon receipt, store kit immediately at 2-8ºC.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 9 Reagent Preparation.

5. MATERIALS SUPPLIED

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
<th>Storage Condition (Before Preparation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microplate coated with polyclonal antibody to Human PMN Elastase</td>
<td>96 wells</td>
<td>2-8 ºC</td>
</tr>
<tr>
<td>(12 x 8 wells)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1X HRP-Conjugate anti-Human alpha1-PI polyclonal antibody</td>
<td>16 mL</td>
<td>2-8 ºC</td>
</tr>
<tr>
<td>Human PMN Elastase Standard, lyophilized</td>
<td>1 Vial</td>
<td>2-8 ºC</td>
</tr>
<tr>
<td>Control high, lyophilized</td>
<td>1 Vial</td>
<td>-20 ºC</td>
</tr>
<tr>
<td>Control low, lyophilized</td>
<td>1 Vial</td>
<td>-20 ºC</td>
</tr>
<tr>
<td>Sample Diluent</td>
<td>50 mL</td>
<td>2-8 ºC</td>
</tr>
<tr>
<td>10X Wash Buffer Concentrate</td>
<td>50 mL</td>
<td>2-8 ºC</td>
</tr>
<tr>
<td>TMB Substrate Solution</td>
<td>22 mL</td>
<td>2-8 ºC</td>
</tr>
<tr>
<td>Stop Solution (2M Hydrochloric acid)</td>
<td>7 mL</td>
<td>2-8 ºC</td>
</tr>
</tbody>
</table>
6. **MATERIALS REQUIRED, NOT SUPPLIED**

These materials are not included in the kit, but will be required to successfully utilize this assay:

- 5 mL and 10 mL graduated pipettes
- 5 µL to 1000 µL adjustable single channel micropipettes with disposable tips
- 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
- Multichannel micropipette reservoir
- Beakers, flasks, cylinders necessary for preparation of reagents
- Device for delivery of wash solution (multichannel wash bottle or automatic wash system)
- Microplate strip reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Glass-distilled or deionized water
- Statistical calculator with program to perform regression analysis

7. **LIMITATIONS**

- Assay kit intended for research use only. Not for use in diagnostic procedures
- Do not use kit or components if it has exceeded the expiration date on the kit labels
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted
8. TECHNICAL HINTS

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps.
- As exact conditions may vary from assay to assay, a standard curve must be established for every run.
- Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.
- Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Empty wells completely before dispensing fresh wash solution, fill with Wash Buffer as indicated for each wash cycle and do not allow wells to sit uncovered or dry for extended periods.
- The use of radio immunotherapy has significantly increased the number of patients with Human anti-mouse IgG antibodies (HAMA). HAMA may interfere with assays utilizing murine monoclonal antibodies leading to both false positive and false negative results. Serum samples containing antibodies to murine immunoglobulins can still be analyzed in such assays when murine immunoglobulins (serum, ascitic fluid, or monoclonal antibodies of irrelevant specificity) are added to the sample.
- **This kit is sold based on number of tests.** A ‘test’ simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
9. **REAGENT PREPARATION**

Equilibrate all reagents and samples to room temperature (18-25°C) prior to use. If crystals have formed in the Buffer Concentrate, warm it gently until they have completely dissolved.

9.1 **1X Wash Buffer**

Prepare 1X Wash Buffer by diluting the 10X Wash Buffer Concentrate with distilled or deionized water. To make 500 mL 1X Wash Buffer for 1 to 12 strips, combine 50 mL 10X Wash Buffer Concentrate with 450 mL distilled or deionized water. For 1-6 Strips make 250 mL 1X Wash Buffer, add 25 mL to 225 mL of water. Mix thoroughly and gently to avoid foaming.

*Note: The 1X Wash Buffer should be stored at 2-8 ºC and is stable for 30 days.*

9.2 **Control - low**

Reconstitute the lyophilized low control by adding 1 mL Sample Diluent to the vial 30 minutes before use. The low control may be used without further dilution. Aliquots of the reconstituted low control may be stored at -20 ºC. Avoid repeated freeze thaw cycles.

9.3 **Control - high**

Reconstitute the lyophilized high control by adding 1 mL Sample Diluent to the vial 30 minutes before use. The high control may be used without further dilution. Aliquots of the reconstituted low control may be stored at -20 ºC. Avoid repeated freeze thaw cycles.
10. STANDARD PREPARATIONS

10.1 Prepare a 10 ng/mL Stock Standard by reconstituting one vial of the PMN Elastase standard with the volume of Sample Diluent stated on the label. Hold at room temperature for 30 minutes. The 10 ng/mL Stock Standard can be aliquoted and stored at -20°C.

10.2 Label eight tubes with numbers 2 - 8.

10.3 Add 225 µL Sample Diluent Buffer into tubes 2 - 8.

10.4 Label the Stock Standard tube as 1.

10.5 Prepare a 5 ng/mL Standard 2 by adding 225 µL of the 10 ng/mL Stock Standard to 225 µL 1X Assay Buffer to tube 1. Mix thoroughly and gently.

10.6 Prepare Standard 3 by transferring 225 µL from Standard 2 to tube 3. Mix thoroughly and gently.

10.7 Using the table below as a guide, repeat for tubes number 4 through to 7.

10.8 Standard 8 contains no protein and is the Blank control.
# ASSAY PREPARATION

<table>
<thead>
<tr>
<th>Standard</th>
<th>Sample to Dilute</th>
<th>Volume to Dilute (µL)</th>
<th>Volume of Diluent (µL)</th>
<th>Starting Conc. (ng/mL)</th>
<th>Final Conc. (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Step 10.1</td>
</tr>
<tr>
<td>2</td>
<td>Standard 1</td>
<td>225</td>
<td>225</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>Standard 2</td>
<td>225</td>
<td>225</td>
<td>5</td>
<td>2.5</td>
</tr>
<tr>
<td>4</td>
<td>Standard 3</td>
<td>225</td>
<td>225</td>
<td>2.5</td>
<td>1.25</td>
</tr>
<tr>
<td>5</td>
<td>Standard 4</td>
<td>225</td>
<td>225</td>
<td>1.25</td>
<td>0.63</td>
</tr>
<tr>
<td>6</td>
<td>Standard 5</td>
<td>225</td>
<td>225</td>
<td>0.63</td>
<td>0.31</td>
</tr>
<tr>
<td>7</td>
<td>Standard 6</td>
<td>225</td>
<td>225</td>
<td>0.31</td>
<td>0.16</td>
</tr>
<tr>
<td>8</td>
<td>None</td>
<td>-</td>
<td>225</td>
<td>-</td>
<td>0</td>
</tr>
</tbody>
</table>

![Diagram of assay preparation](image)
11. SAMPLE COLLECTION AND STORAGE

- Cell culture supernatants, plasma, exudate, bronchoalveolar lavage fluid, cerebrospinal fluid and seminal plasma were tested with this assay. Other biological samples might be suitable for use in the assay. Remove serum or plasma from the clot or cells as soon as possible after clotting and separation.

- Possible “Hook Effects” may be observed due to high sample concentrations. It is recommended to run several dilutions of your sample to ensure an accurate reading.

- Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens.

- Samples should be aliquoted and must be stored frozen at -20°C to avoid loss of bioactive Human PMN Elastase. If samples are to be run within 24 hours, they may be stored at 2° to 8°C.

- Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.
12. **PLATE PREPARATION**

- The 96 well plate strips included with this kit are supplied ready to use.
- Unused well strips should be returned to the plate packet and stored at 2-8°C.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Well effects have not been observed with this assay.
13. ASSAY PROCEDURE

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- It is recommended to assay all standards, controls and samples in duplicate.

13.1. Prepare all reagents, working standards, and samples as directed in the previous sections. Determine the number of microplate strips required to test the desired number of samples plus appropriate number of wells needed for running blanks and standards.

13.2. Predilute samples before beginning the test procedure. Dilute samples 1:100 with Sample Diluent as follows:
   13.2.1. Dilution 1: 10 µL + 90 µL Sample Diluent
   13.2.2. Dilution 2: 50 µL Dilution 1 + 450 µL Sample Diluent

13.3. Wash the microplate twice with approximately 400 µL 1X Wash Buffer per well with thorough aspiration of microplate contents between washes. Allow the 1X Wash Buffer to remain in the wells for about 10 - 15 seconds before aspiration. Take care not to scratch the surface of the microplate.

13.4. After the last wash step, empty wells and tap microplate on absorbent pad or paper towel to remove excess 1X Wash Buffer. Use the microplate strips immediately after washing. Alternatively the microplate strips can be placed upside down on a wet absorbent paper for not longer than 15 minutes. Do not allow wells to dry.

13.5. Add 100 µL of the prepared standards to appropriate wells.

13.6. Add 100 µL of each sample or High or Low controls to appropriate wells.

13.7. Cover with adhesive film and incubate at room temperature (18° to 25°C) for 1 hour (microplate can be incubated on a shaker set at 400 rpm).

13.9. Add 150 µL of 1X HRP Conjugated Antibody to all wells.

13.10. Cover with adhesive film and incubate at room temperature (18° to 25°C) for 1 hour (microplate can be incubated on a shaker set at 400 rpm).


13.12. Add 200 µL of TMB Substrate Solution to all wells.

13.13. Incubate the microplate strips at room temperature (18 to 25°C) for 20 minutes. Avoid direct exposure to intense light.

Note: The color development on the plate should be monitored and the substrate reaction stopped (see step 13.14) before the signal in the positive wells becomes saturated. Determination of the ideal time period for color development should to be done individually for each assay. It is recommended to add the stop solution when the highest standard has developed a dark blue color. Alternatively the color development can be monitored by the ELISA reader at 620 nm. The substrate reaction should be stopped as soon as Standard 1 has reached an OD of 0.9 - 0.95.

13.14. Stop the enzyme reaction by adding 50 µL of Stop Solution into each well.

Note: It is important that the Stop Solution is mixed quickly and uniformly throughout the microplate to completely inactivate the enzyme. Results must be read immediately after the Stop Solution is added or within one hour if the microplate strips are stored at 2 - 8°C in the dark.

13.15. Read absorbance of each microplate on a spectrophotometer using 450 nm as the primary wave
length (optionally 620 nm as the reference wavelength; 610 nm to 650 nm is acceptable). Blank the plate reader according to the manufacturer's instructions by using the blank wells. Determine the absorbance of both the samples and the standards.

*Note:* In case of incubation without shaking the obtained O.D. values may be lower than indicated below. Nevertheless the results are still valid.
14. CALCULATIONS

Average the duplicate reading for each standard, sample and control blank. Subtract the control blank from all mean readings. Plot the mean standard readings against their concentrations and draw the best smooth curve through these points to construct a standard curve. Most plate reader software or graphing software can plot these values and curve fit. A five parameter algorithm (5PL) usually provides the best fit, though other equations can be examined to see which provides the most accurate (e.g. linear, semi-log, log/log, 5-parameter logistic). Extrapolate protein concentrations for unknown and control samples from the standard curve plotted. Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.

If samples have been diluted 1:100, as stated in step 13.2, the concentration obtained from the standard curve must be multiplied by the dilution factor (x 100) to obtain an accurate value. This is in addition to any sample dilution used by the user.

If controls have not been diluted, the concentration obtained from the standard curve must be multiplied by the dilution factor (x 1) to obtain an accurate value.

Calculation of 1:100 prediluted samples with a concentration exceeding standard 1 may result in incorrect, low Human PMN Elastase levels (Hook Effect). Such samples require further external predilution according to expected Human PMN Elastase values with Sample Diluent in order to precisely quantitate the actual Human PMN Elastase level.
15. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

Figure 1. Example of Human PMN Elastase protein standard curve.

<table>
<thead>
<tr>
<th>Conc. (ng/mL)</th>
<th>O.D. 450 nm 1</th>
<th>O.D. 450 nm 2</th>
<th>Mean O.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>0.048</td>
<td>0.046</td>
<td>0.047</td>
</tr>
<tr>
<td>0.16</td>
<td>0.226</td>
<td>0.210</td>
<td>0.218</td>
</tr>
<tr>
<td>0.31</td>
<td>0.377</td>
<td>0.356</td>
<td>0.366</td>
</tr>
<tr>
<td>0.63</td>
<td>0.629</td>
<td>0.598</td>
<td>0.613</td>
</tr>
<tr>
<td>1.25</td>
<td>1.005</td>
<td>1.001</td>
<td>1.003</td>
</tr>
<tr>
<td>2.50</td>
<td>1.548</td>
<td>1.354</td>
<td>1.541</td>
</tr>
<tr>
<td>5.00</td>
<td>2.184</td>
<td>2.199</td>
<td>2.192</td>
</tr>
<tr>
<td>10.00</td>
<td>2.452</td>
<td>2.579</td>
<td>2.516</td>
</tr>
</tbody>
</table>
16. **TYPICAL SAMPLE VALUES**

**SENSITIVITY -**
The limit of detection of Human PMN Elastase defined as the analyte concentration resulting in an absorbance significantly higher than that of the dilution medium (mean plus 2 standard deviations) was determined to be 1.98 pg/ml (mean of 6 independent assays).

**RECOVERY**
The spike recovery was evaluated by spiking 3 levels of Human PMN Elastase into a plasma sample. Recoveries were determined in 3 independent experiments with 4 replicates each. The unspiked plasma was used as blank in these experiments. The recovery ranged from 96% to 110% with an overall mean recovery of 104%.

**LINEARITY OF DILUTION –**
Plasma samples with different levels of Human PMN Elastase were analyzed at serial 2 fold dilutions with 4 replicates each. The recovery ranged from 87% to 114% with an overall recovery of 96.5%.

**EXPECTED VALUES –**
A panel of 57 plasma samples from randomly selected apparently healthy donors (males and females) was tested for Human PMN elastase. The detected Human PMN elastase mean level was 35 ng/ml. The levels measured may vary with the sample collection used.

**PRECISION –**
Intra- and Inter-assay reproducibility was determined by measuring samples containing different concentrations of Human PMN Elastase.

<table>
<thead>
<tr>
<th></th>
<th>Intra-Assay</th>
<th>Inter-Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>%CV</td>
<td>4.8</td>
<td>5.6</td>
</tr>
</tbody>
</table>
## 17. TROUBLESHOOTING

<table>
<thead>
<tr>
<th>Problem</th>
<th>Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor standard curve</td>
<td>Inaccurate pipetting</td>
<td>Check pipettes</td>
</tr>
<tr>
<td></td>
<td>Improper standards dilution</td>
<td>Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing</td>
</tr>
<tr>
<td>Low Signal</td>
<td>Incubation times too brief</td>
<td>Ensure sufficient incubation times; change to overnight standard/sample incubation</td>
</tr>
<tr>
<td></td>
<td>Inadequate reagent volumes or improper dilution</td>
<td>Check pipettes and ensure correct preparation</td>
</tr>
<tr>
<td>Samples give higher value than the highest standard</td>
<td>Starting sample concentration is too high.</td>
<td>Dilute the specimens and repeat the assay</td>
</tr>
<tr>
<td>Large CV</td>
<td>Plate is insufficiently washed</td>
<td>Review manual for proper wash technique. If using a plate washer, check all ports for obstructions</td>
</tr>
<tr>
<td></td>
<td>Contaminated wash buffer</td>
<td>Prepare fresh wash buffer</td>
</tr>
<tr>
<td>Low sensitivity</td>
<td>Improper storage of the kit</td>
<td>Store the all components as directed.</td>
</tr>
</tbody>
</table>
18. **NOTES**