ab119553 PMN Elastase Human ELISA Kit

For the quantitative measurement of Human PMN Elastase concentrations in Cell culture supernatants, plasma, serum, exudate, bronchoalveolar lavage fluid, cerebrospinal fluid and seminal plasma. This product is for research use only and is not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab119553
(use abcam.cn/ab119553 for China, or abcam.co.jp/ab119553 for Japan)

Materials Supplied and Storage

Store kit at +4°C immediately upon receipt, apart from the Control high and Control low which should be stored at -20°C. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.
Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Item	Quantity	Storage Condition
Microplate coated with polyclonal antibody to Human PMN Elastase (12 x 8 wells)	96 wells	4°C
1X HRP-Conjugate anti-Human alpha1-PI polyclonal antibody	16 mL	4°C
Human PMN Elastase - alpha1-PI complex Standard, lyophilized	1 vial	4°C
Control high, lyophilized	1 vial	-20°C
Control low, lyophilized	1 vial	-20°C
Sample Diluent	50 mL	4°C
10X Wash Buffer Concentrate	50 mL	4°C
TMB Substrate Solution	22 mL	4°C
Stop Solution (2M Hydrochloric acid)	7 mL	4°C

Materials Required, Not Supplied

- 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

1. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. Prepare only as much reagent as is needed on the day of the experiment.

1.1 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.
Δ Note: The 1X Wash Buffer should be stored at 4°C and is stable for 30 days.

1.2 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 °C. Avoid repeated freeze thaw cycles.

1.3 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 °C. Avoid repeated freeze thaw cycles

2. Standard Preparation

Prepare a fresh set of standards for every use. Discard working standard dilutions after use. The following section describes the preparation of a standard curve for duplicate measurements (recommended).

2.1 Prepare a 10 ng/mL **Stock Standard** by reconstituting one vial of the PMN Elastase – alpha-P1 complex standard in 2000 µL Sample Diluent. Hold at room temperature for 30 minutes. The 10 ng/mL **Stock Standard** can be aliquoted and stored at -20°C.
2.2 Label eight tubes with numbers 1-8.
2.3 Add 225 µL Sample Diluent Buffer into tubes 2 - 8.
2.4 Label the **Stock Standard** tube as **#1**.
2.5 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.
2.6 Prepare **Standard #3** by transferring 225 µL from Standard 2 to tube 3. Mix thoroughly and gently.
2.7 Using the table below as a guide, repeat for tubes number #4 through to #7.
2.8 **Standard #8** contains no protein and is the Blank control.

Standard #	Sample to Dilute	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting conc. (ng/mL)	Final conc. (ng/mL)
1	Step 2.1				10
2	Standard #1	225	225	10	5
3	Standard #2	225	225	5	2.5
4	Standard #3	225	225	2.5	1.25
5	Standard #4	225	225	1.25	0.63
6	Standard #5	225	225	0.63	0.31
7	Standard #6	225	225	0.31	0.16
8 (Blank)	None	-	225	-	0

3. Sample Preparation

- Cell culture supernatants, plasma, serum, 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.

Guidelines for Dilutions of 100-fold or Greater *(for reference only)*	
100x	**10000x**
4 µl sample + 396 µl buffer (100X) = 100-fold dilution *Assuming the needed volume is less than or equal to 400 µl*	A) 4 µl sample + 396 µl buffer (100X) B) 4 µl of A + 396 µl buffer (100X) = 10000-fold dilution *Assuming the needed volume is less than or equal to 400 µl*
1000x	**100000x**
A) 4 µl sample + 396 µl buffer (100X) B) 24 µl of A + 216 µl buffer (10X) = 1000-fold dilution *Assuming the needed volume is less than or equal to 240 µl*	A) 4 µl sample + 396 µl buffer (100X) B) 4 µl of A + 396 µl buffer (100X) C) 24 µl of A + 216 µl buffer (10X) = 100000-fold dilution *Assuming the needed volume is less than or equal to 240 µl*

4. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.

4.1 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.

4.2 Predilute samples before beginning the test procedure. Dilute samples 1:100 with Sample Diluent as follows:
Dilution 1: 10 µL + 90 µL Sample Diluent
Dilution 2: 50 µL Dilution 1 + 450 µL Sample Diluent
Δ Note: Optimal dilutions will need to be determined for each sample type. A range of 1:10 – 1:100 for plasma and serum, and 1:100 – 1:1000 for CSF, was found to be best.

4.3 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.

4.4 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 out.

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

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

4.7 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).

4.8 Remove adhesive film and empty wells. Wash microplate strips 4 times according to step 4.3. Proceed immediately to step 4.9.

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

4.10 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).

4.11 Remove adhesive film and empty wells. Wash microplate strips 4 times according to step 4.3. Proceed immediately to step 4.12.

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

4.13 Incubate the microplate strips at room temperature (18 to 25°C) for 20 minutes. Avoid direct exposure to intense light.
Δ Note: The color development on the plate should be monitored and the substrate reaction stopped (see step 4.14) 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.

4.14 Stop the enzyme reaction by adding 50 µL of Stop Solution into each well.
Δ 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 4°C in the dark.

4.15 Read absorbance of each microplate on a spectrophotometer using 450 nm as the primary wave length (optionally 620 nm as the reference wave length; 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.

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

5. Calculations

5.1 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.

5.2 If samples have been diluted 1:100, as stated in step 4.2, 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.

5.3 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.

5.4 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.

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