ELISA PRODUCT INFORMATION & MANUAL

Human Neutrophil Elastase/ ELA2 ELISA Kit (Colorimetric)

NBP1-91266

Enzyme-linked Immunosorbent Assay for quantitative detection. For research use only.
Not for diagnostic or therapeutic procedures.

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Human Neutrophil Elastase/ELA2 ELISA Kit

Enzyme-linked Immunosorbent Assay for quantitative detection of human Neutrophil Elastase/ELA2

Catalog Number NBP1-91266

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from Technical Support.

Product description

The Human Neutrophil Elastase/ELA2 ELISA Kit is an enzyme-linked immunosorbent assay for the quantitative detection of human Neutrophil Elastase/ELA2.

Summary

The human organism reacts with an in lammatory 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 in lammatory reaction. Different bloodstream mediators (cytokines, leukotrienes, complement factors, bacterial endotoxins, clotting and ibrinolysis 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 α 1-proteinase inhibitor, α 1-PI). An overwhelming release of PMN elastase, however, can exceed the inhibitory potential of the α 1-proteinase inhibitor. Thus, enzymatically active PMN elastase, together with simultaneously produced oxidants (O2-radicals, H2O2, OH-radicals), can cause local tissue injury.

Principles of the test

An anti-human Neutrophil Elastase/ELA2 coating antibody is adsorbed onto microwells.

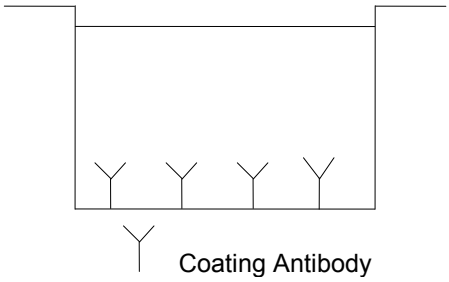

Fig. 1 Coated microwell

Human Neutrophil Elastase/ELA2 present in the sample or standard binds to antibodies adsorbed to the microwells.

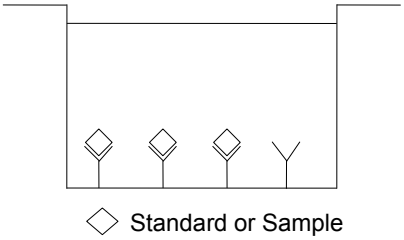

Fig. 2 First incubation

Following incubation unbound biological components are removed during a wash step and a HRP-conjugated anti-α 1-PI antibody is added and binds to human Neutrophil Elastase/ELA2/α 1-PI complex captured by the first antibody.

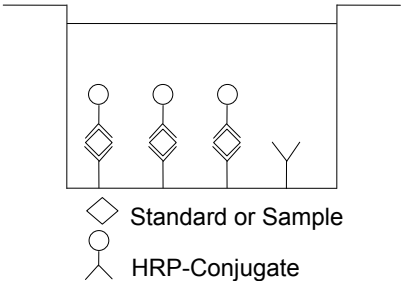

Fig. 3 Second incubation

Following incubation unbound HRP-conjugated anti-α 1-PI antibody is removed during a wash step, and substrate solution reactive with HRP is added to the wells.

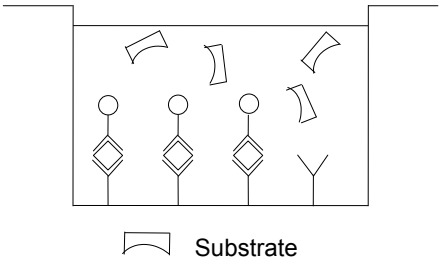

Fig. 4 Third incubation

A colored product is formed in proportion to the amount of human Neutrophil Elastase/ELA2 present in the sample or standard. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from 7 human Neutrophil Elastase/ELA2 standard dilutions and human Neutrophil Elastase/ELA2 concentration determined.

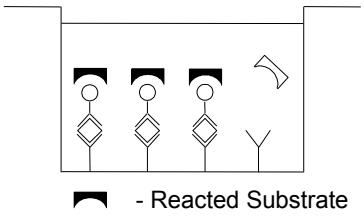

Fig. 5 Stop reaction

Reagents provided

1 aluminum pouch with a Microwell Plate (12 strips of 8 wells each) coated with polyclonal antibody to human Neutrophil Elastase/ELA2

1 vial (16 mL) HRP-Conjugate anti-α 1-PI polyclonal antibody, ready to use

1 vial human Neutrophil Elastase/ELA2 Standard lyophilized, 10 ng/mL upon reconstitution

1 vial Control high, lyophilized

1 vial Control low, lyophilized

1 bottle (50 mL) Sample Diluent

1 bottle (50 mL) Wash Buffer Concentrate (10x)

1 vial (22 mL) Substrate Solution (tetramethyl-benzidine)

1 vial (7 mL) Stop Solution (2M hydrochloric acid)

2 Adhesive Films

Storage instructions – ELISA kit

Store kit reagents between 2° and 8°C except controls. Store lyophilized controls at –20°C. Immediately after use remaining reagents should be returned to cold storage (2–8°C), or to –20°C, respectively. Expiry of the kit and reagents is stated on labels.

Expiry of the kit components can be guaranteed only if the components are stored properly, and if, in case of repeated use of one component, this reagent is not contaminated by the first handling.

Sample collection and storage instructions

Cell culture supernatants, plasma, exudate, bronchoalveolar lavage fluid, cerebrospinal fluid, and seminal plasma were tested with this assay. Other body luids might be suitable for use in the assay. Separate plasma from cells by centrifugation.

Pay attention to a possible *Hook Effect* due to high sample concentrations (see chapter "Calculation of results" on page 4).

Samples containing a visible precipitate must be clari ied prior to use in the assay. Do not use grossly hemolyzed or lipemic samples.

Samples should be aliquoted and must be stored frozen at –20°C to avoid loss of bioactive human Neutrophil Elastase/ELA2.

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

Materials required but not provided

- 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)
- Microwell 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

Precautions for use

- All reagents should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses, and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statement(s) for specific advice.
- Reagents are intended for research use only and are not for use in diagnostic or therapeutic procedures.
- Do not mix or substitute reagents with those from other lots or other sources.
- Do not use kit reagents beyond expiration date on label.
- Do not expose kit reagents to strong light during storage or incubation.
- Do not pipet by mouth.
- Do not eat or smoke in areas where kit reagents or samples are handled.
- Avoid contact of skin or mucous membranes with kit reagents or samples.
- Rubber or disposable latex gloves should be worn while handling kit reagents or samples.
- Avoid contact of substrate solution with oxidizing agents and metal.
- Avoid splashing or generation of aerosols.
- To avoid microbial contamination or cross-contamination of reagents or samples that may invalidate the test, use disposable pipette tips and/or pipettes.
- Use clean, dedicated reagent trays for dispensing the conjugate and substrate reagent.
- Exposure to acid inactivates the conjugate.
- Glass-distilled water or deionized water must be used for reagent preparation.
- Substrate solution must be at room temperature prior to use.
- Decontaminate and dispose samples and all potentially contaminated materials as if they could contain infectious agents. The preferred method of decontamination is autoclaving for a minimum of 1 hour at 121.5°C.
- Liquid wastes not containing acid and neutralized waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 1.0% sodium hypochlorite. Allow 30 minutes for effective decontamination. Liquid waste containing acid must be neutralized prior to the addition of sodium hypochlorite.

Preparation of reagents

1. Buffer Concentrate should be brought to room temperature and should be diluted before starting the test procedure.
2. If crystals have formed in the Buffer Concentrate, warm it gently until they have completely dissolved.

Wash buffer (1x)

1. Pour entire contents (50 mL) of the Wash Buffer Concentrate (10x) into a clean 1000 mL graduated cylinder. Bring to final volume of 500 mL with glass-distilled or deionized water. Mix gently to avoid foaming.
2. Transfer to a clean wash bottle and store at 2° to 25°C. Please note that Wash Buffer (1x) is stable for 30 days.
3. Wash Buffer (1x) may also be prepared as needed according to the following table:

Number of Strips	Wash Buffer Concentrate (10x) (mL)	Distilled Water (mL)
1 - 6	25	225
1 - 12	50	450

Human Neutrophil Elastase/ELA2 standard

1. Reconstitute human Neutrophil Elastase/ELA2 standard by addition of Sample Diluent 30 minutes before use. Reconstitution volume is stated on the label of the standard vial. Swirl or mix gently to insure complete and homogeneous solubilisation (concentration of reconstituted standard = 10 ng/mL).
2. Aliquots can be stored at –20°C.
3. Standard dilutions can be prepared directly on the microwell plate (see "Test protocol" on page 3) or alternatively in tubes (see "External standard dilution" on page 3).

External standard dilution

1. Label 6 tubes, one for each standard point: S2, S3, S4, S5, S6, S7.
2. Prepare 1:2 serial dilutions for the standard curve as follows: Pipette 225 µL of Sample Diluent into each tube.
3. Pipette 225 µL of reconstituted (serves as the highest standard S1, concentration of standard 1 = 10 ng/mL) into the first tube, labeled S2, and mix (concentration of standard 2 = 5 ng/mL).
4. Pipette 225 µL of this dilution into the second tube, labeled S3, and mix thoroughly before the next transfer.
5. Repeat serial dilutions 4 more times thus creating the points of the standard curve (see Figure 6).

   Sample Diluent serves as blank.

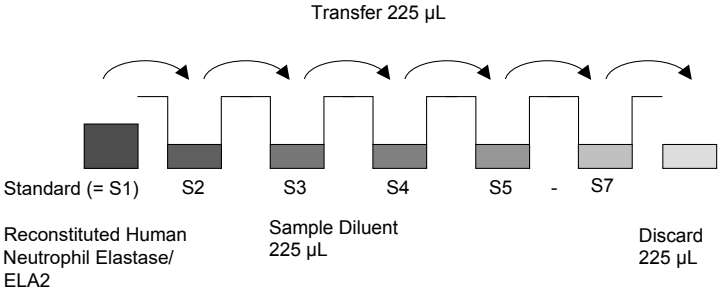

Fig. 6 Dilute standards - tubes

Controls

Reconstitute by adding 1 mL Sample Diluent to lyophilized controls 30 minutes before use. No further dilution necessary. For control range please refer to certificate of analysis or vial label. Store reconstituted controls aliquoted at -20°C. Avoid repeated freeze and thaw cycles.

Test protocol

1. Predilute your samples before starting with the test procedure. Dilute samples 1:100 with Sample Diluent according to the following scheme:

   Dilution 1: 10 µL sample + 90 µL Sample Diluent

   Dilution 2: 50 µL of dilution 1 + 450 µL Sample Diluent
2. Determine the number of microwell strips required to test the desired number of samples plus appropriate number of wells needed for running blanks and standards. Each sample, standard, blank ans optional control sample should be assayed in duplicate. Remove extra microwell strips from holder and store in foil bag with the desiccant provided at 2°-8°C sealed tightly.
3. Standard dilution on the microwell plate (Alternatively the standard dilution can be prepared in tubes - see "External standard dilution" on page 3): Add 100 µL of Sample Diluent in duplicate to to standard wells B1/2-G1/2, leaving A1/A2 empty. Pipette 200 µL of prepared standard (see Preparation of Standard "Human Neutrophil Elastase/ELA2 standard" on page 3, concentration of S1 = 10.00 ng/mL) in duplicate into well A1 and A2 (see Table 1). Transfer 100 µL to wells B1 and B2. Mix the contents of wells B1 and B2 by repeated aspiration and ejection (concentration of standard, S2 = 5.00 ng/mL), and transfer 100 µL to wells C1 and C2, respectively (see Figure 7). Take care not to scratch the inner surface of the microwells. Continue this procedure 4 times, creating two rows of human Neutrophil Elastase/ELA2 standard dilutions ranging from 10.00 to 0.16 ng/mL. Discard 100 µL of the contents from the last microwells (G1, G2) used.

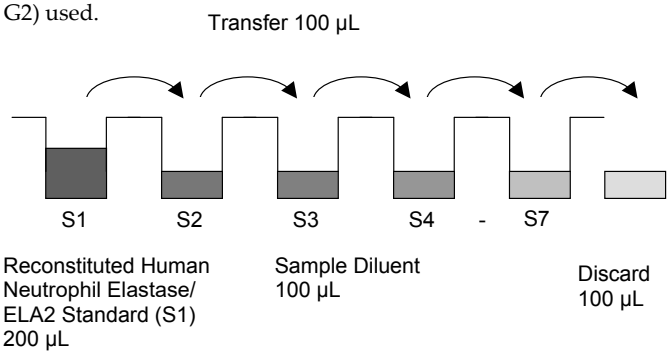

Fig. 7 Dilute standards - microwell plate

Table 1 Example of the arrangement of blanks, standards and samples in the microwell strips.

	1	2	3	4
A	Standard 1 10.00 ng/mL	Standard 1 10.00 ng/mL	Sample 1	Sample 1
B	Standard 2 5.00 ng/mL	Standard 2 5.00 ng/mL	Sample 2	Sample 2
C	Standard 3 2.50 ng/mL	Standard 3 2.50 ng/mL	Sample 3	Sample 3
D	Standard 4 1.25 ng/mL	Standard 4 1.25 ng/mL	Sample 4	Sample 4
E	Standard 5 0.63 ng/mL	Standard 5 0.63 ng/mL	Sample 5	Sample 5
F	Standard 6 0.31 ng/mL	Standard 6 0.31 ng/mL	Sample 6	Sample 6
G	Standard 7 0.16 ng/mL	Standard 7 0.16 ng/mL	Sample 7	Sample 7
H	Blank	Blank	Sample 8	Sample 8

   In case of an external standard dilution (see "External standard dilution" on page 3), pipette 100 µL of these standard dilutions (S1 – S7) in the standard wells according to Table 1.
4. Add 100 µL of Sample Diluent in duplicate to the blank wells.
5. Add 100 µL of each prediluted sample/reconstituted controls (not prediluted) in duplicate to the sample wells.
6. Cover with an adhesive film and incubate at room temperature (18 to 25°C) for 1 hour on a microplate shaker.
7. Remove adhesive film and empty wells. Wash the microwell strips 4 times with approximately 400 µL Wash Buffer per well with thorough aspiration of microwell contents between washes. Allow the Wash Buffer to sit in the wells for about 10 – 15 seconds before aspiration. Take care not to scratch the surface of the microwells.

   After the last wash step, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffer. Use the microwell strips immediately after washing. Do not allow wells to dry.
8. Add 150 µL of HRP-Conjugate, ready to use to all wells.
9. Cover with an adhesive film and incubate at room temperature (18 to 25°C) for 1 hour on a microplate shaker.

10. Remove adhesive film and empty wells. Wash microwell strips 4 times according to point 7. of the test protocol. Proceed immediately to the next step.
11. Pipette 200 µL of TMB Substrate Solution to all wells.
12. Incubate the microwell strips at room temperature (18° to 25°C) for about 20 minutes. Avoid direct exposure to intense light.

    The color development on the plate should be monitored and the substrate reaction stopped (see next point of this protocol) before positive wells are no longer properly recordable. Determination of the ideal time period for color development has 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. Stop the enzyme reaction by quickly pipetting 50 µL of Stop Solution into each well. It is important that the Stop Solution is spread quickly and uniformly throughout the microwells to completely inactivate the enzyme. Results must be read immediately after the Stop Solution is added or within one hour if the microwell strips are stored at 2 - 8°C in the dark.
14. Read absorbance of each microwell on a spectro-photometer 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.

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

Calculation of results

- Calculate the average absorbance values for each set of duplicate standards and samples. Duplicates should be within 20 percent of the mean value.
- Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the human Neutrophil Elastase/ELA2 concentration on the abscissa. Draw a best it curve through the points of the graph (a 5-parameter curve it is recommended).
- To determine the concentration of circulating human Neutrophil Elastase/ELA2 for each sample, first fsigni icantind the mean absorbance value on the ordinate and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the abscissa and read the corresponding human Neutrophil Elastase/ELA2 concentration.
- If instructions in this protocol have been followed, samples have been diluted 1:100 and the concentration read from the standard curve must be multiplied by the dilution factor (x 100).
- If instructions in this protocol have been followed, controls have not been diluted and the concentration read from the standard curve must be multiplied by the dilution factor (x 1).
- Calculation of 1:100 prediluted samples with a concentration exceeding standard 1 may result in incorrect, low human Neutrophil Elastase/ELA2 levels (Hook Effect). Such samples require further external predilution according to expected human Neutrophil Elastase/ELA2 values with Sample Diluent in order to precisely quantitate the actual human Neutrophil Elastase/ELA2 level.
- It is suggested that each testing facility establishes a control sample of known human Neutrophil Elastase/ELA2 concentration and runs this additional control with each assay. If the values obtained are not within the expected range of the control, the assay results may be invalid.
- A representative standard curve is shown in Figure 8.

  Note: Do not use this standard curve to derive test results. Each laboratory must prepare a standard curve for each group of microwell strips assayed.

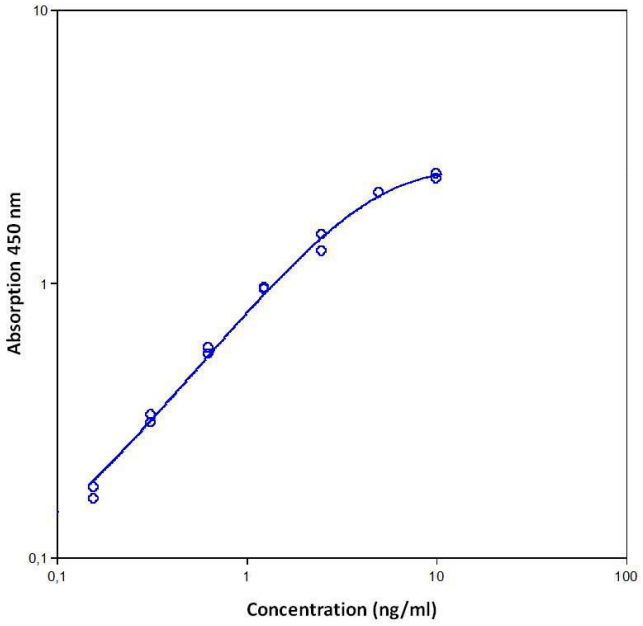

Fig. 8 Representative standard curve for human Neutrophil Elastase/ ELA2 ELISA. Human Neutrophil Elastase/ELA2 was diluted in serial 2-fold steps in Sample Diluent.

Table 2 Typical data using the human Neutrophil Elastase/ELA2 ELISA.
Measuring wavelength: 450 nm
Reference wavelength: 620 nm

Standard	Human Neutrophil Elastase/ELA2 Concentration (ng/mL)	O.D. at 450 nm	Mean O.D. at 450 nm	C.V. (%)
1	10.00	2.452 2.579	2.516	2.5
2	5.00	2.184 2.199	2.192	0.3
3	2.50	1.548 1.354	1.451	6.7
4	1.25	1.005 1.001	1.003	0.2
5	0.63	0.629 0.598	0.613	2.6
6	0.31	0.377 0.356	0.366	2.8
7	0.16	0.226 0.210	0.218	3.6
Blank	0	0.048 0.046	0.047	3.3

The OD values of the standard curve may vary according to the conditions of assay performance (e.g., operator, pipetting technique, washing technique, or temperature effects). Furthermore, shelf life of the kit may affect enzymatic activity and thus color intensity. Values measured are still valid.

Limitations

- Since exact conditions may vary from assay to assay, a standard curve must be established for every run.
- Bacterial or fungal contamination of either screen samples or reagents or cross-contamination between reagents may cause erroneous results.

- 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.

Performance characteristics

Sensitivity

The limit of detection of human Neutrophil Elastase/ELA2 de ined as the analyte concentration resulting in an absorbance signi icantly 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).

Reproducibility

Intra-assay

Reproducibility within the assay was evaluated in 10 independent experiments. Each assay was carried out with 10 replicates of 3 plasma samples containing different concentrations of human Neutrophil Elastase/ELA2. 2 standard curves were run on each plate. The calculated overall intra-assay coefficient of variation was 4.8%.

Inter-assay

Assay to assay reproducibility within one laboratory was evaluated in 10 independent experiments. Each assay was carried out with 10 replicates of 4 plasma samples containing different concentrations of human Neutrophil Elastase/ELA2. 2 standard curves were run on each plate. The calculated overall inter-assay coefficient of variation was 5.6%.

Spike recovery

The spike recovery was evaluated by spiking 3 levels of human Neutrophil Elastase/ELA2 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%.

Sample	Spiking Solution	Expected Neutrophil Elastase/ELA2 Concentration (ng/mL)	Observed Neutrophil Elastase/ELA2 Concentration (ng/mL)	Recovery of Expected Neutrophil Elastase/ELA2 Concentration (%)
1	-	-	23.2	-
	A	69.4	72.4	104
	B	54.1	59.3	109
	C	47.2	49.6	101
2	-	-	30.6	-
	A	76.7	73.4	96
	B	61.4	59.3	97
	C	54.4	56.8	104
3	-	-	61.7	-
	A	107.8	118.0	109
	B	92.5	100.8	109
	C	85.6	94.8	110

Dilution parallelism

Plasma samples with different levels of human Neutrophil Elastase/ELA2 were analyzed at serial 2-fold dilutions with 4 replicates each.

The recovery ranged from 87–114% with an overall recovery of 96.5%.

Sample	Dilution	Neutrophil Elastase/ELA2 (ng/mL)		Recovery of expected concentration (%)
		Expected concentration	Observed concentration	
1	1:100	–	114.0	–
	1:200	55.7	57.9	103
	1:400	27.8	31.8	114
	1:800	13.9	14.6	105
2	1:100	–	135.6	–
	1:200	67.8	66.5	98
	1:400	33.9	30.8	91
	1:800	16.9	18.8	111
3	1:100	–	255.0	–
	1:200	127.5	130.5	102
	1:400	63.8	61.0	96
	1:800	31.9	31.9	100
4	1:100	–	540.1	–
	1:200	270.0	246.2	92
	1:400	135.0	119.9	89
	1:800	67.5	61.7	91
5	1:100	–	641.8	–
	1:200	320.9	281.4	88
	1:400	160.4	149.7	93
	1:800	80.2	69.9	87
6	1:100	–	909.5	–
	1:200	454.7	444.5	98
	1:400	227.4	208.1	92
	1:800	113.7	100.4	88

Specificity

The cross-reactivity and interference of circulating factors of the immune system was evaluated by spiking these proteins at physiologically relevant concentrations into a human Neutrophil Elastase/ELA2 positive sample. No cross-reactivity or interference wasdetected.

Expected values

A panel of 57 plasma samples from randomly selected apparently healthy donors (males and females) was tested for human Neutrophil Elastase/ELA2. The detected human Neutrophil Elastase/ELA2 mean level was 35 ng/mL. The levels measured may vary with the sample collection used.

Reagent preparation summary

Wash buffer (1x)

Add Wash Buffer Concentrate 10x (50 mL) to 450 mL distilled water.

Number of Strips	Wash Buffer Concentrate (10x) (mL)	Distilled Water (mL)
1 - 6	25	225
1 - 12	50	450

Human Neutrophil Elastase/ELA2 standard

Reconstitute lyophilized human Neutrophil Elastase/ELA2 standard with Sample Diluent 30 minutes before use. (Reconstitution volume is stated on the label of the standard vial.)

Controls

Add 1 mL Sample Diluent to lyophilized controls.

Test protocol summary

1. Predilute sample with Sample Diluent 1:100.
2. Determine the number of microwell strips required.

3. Standard dilution on the microwell plate: Add 100 µL Sample Diluent, in duplicate, to all standard wells leaving the first wells empty. Pipette 200 µL prepared standard into the first wells and create standard dilutions by transferring 100 µL from well to well. Discard 100 µL from the last wells.

   Alternatively external standard dilution in tubes (see "External standard dilution" on page 3): Pipette 100 µL of these standard dilutions in the microwell strips.
4. Add 100 µL Sample Diluent, in duplicate, to the blank wells.
5. Add 100 µL prediluted sample/reconstituted controls (not prediluted) in duplicate, to designated sample wells.
6. Cover microwell strips and incubate 1 hour at room temperature (18° to 25°C).
7. Empty and wash microwell strips 4 times with Wash Buffer.
8. Add 150 µL HRP-Conjugate to all wells.
9. Cover microwell strips and incubate 1 hour at room temperature (18° to 25°C).
10. Empty and wash microwell strips 4 times with Wash Buffer.
11. Add 200 µL of TMB Substrate Solution to all wells.
12. Incubate the microwell strips for about 20 minutes at room temperature (18° to 25°C).
13. Add 50 µL Stop Solution to all wells.
14. Blank microwell reader and measure color intensity at 450 nm.

Note: If instructions in this protocol have been followed, samples have been diluted 1:100 and the concentration read from the standard curve must be multiplied by the dilution factor (x 100).

Note: If instructions in this protocol have been followed, controls have not been diluted and the concentration read from the standard curve must be multiplied by the dilution factor (x1).

Customer and technical support

Visit **https://www.novusbio.com/support** for service and support information.

Limited product warranty

Novus Biologicals and/or its affiliate(s) warrant their products as set forth in Novus Biologicals' Terms and Conditions.
If you have any questions, please contact Novus Biologicals at **https://www.novusbio.com/support**.