Product Manual

Revision date: 31/Jul/2017

Human Neutrophil Elastase Antibody (NELA) ELISA Kit

Catalog No.: abx351279

Size: 96T

Range: 7.813 ng/ml - 500 ng/ml

Sensitivity: 4.688 ng/ml

Storage: Store at 4°C for up to 6 months.

Application: For quantitative detection of NELA in Human Serum, Plasma, Tissue Homogenates and other biological fluids.

Introduction: Neutrophil elastase (EC 3.4.21.37, leukocyte elastase, ELANE, ELA2, elastase 2, neutrophil, elaszym, serine elastase, subtype human leukocyte elastase (HLE)) is a serine proteinase in the same family as chymotrypsin and has broad substrate specificity. Secreted by neutrophils and macrophages during inflammation, it destroys bacteria and host tissue. It also localizes to Neutrophil extracellular traps (NETs), via its high affinity for DNA, an unusual property for serine proteases. As with other serine proteinases it contains a charge relay system composed of the catalytic triad of histidine, aspartate, and serine residues that are dispersed throughout the primary sequence of the polypeptide but that are brought together in the three dimensional conformation of the folded protein. The gene encoding neutrophil elastase, ELA2, consists of five exons. Neutrophil elastase is closely related to other cytotoxic immune serine proteases, such as the granzymes and cathepsin G. It is more distantly related to the digestive CELA1. The neutrophil form of elastase is 218 amino acids long, with two asparagine-linked carbohydrate chains (see glycosylation). It is present in azurophil granules in the neutrophil cytoplasm. There appear to be two forms of neutrophil elastase, termed IIa and IIb.

Principle of the Assay

This kit is based on enzyme-linked immuno-sorbent assay technology. An antigen specific to NELA is pre-coated onto a 96-well plate. The standards and samples are added to the wells and incubated. A biotinylated detection antigen specific to NELA is used as detection reagent. Next, HRP conjugated reagent is added and unbound conjugates are washed away. TMB substrate is used to visualize HRP. TMB is catalyzed by HRP to produce a blue color product that changes into yellow after adding stop solution. The intensity of the color yellow is proportional to the NELA amount bound on the plate. The O.D. absorbance is measured spectrophotometrically at 450 nm in a microplate reader, and then the concentration of NELA can be calculated.

Kit components

1. One pre-coated 96 well plate
2. Standard: 2 tubes
3. Sample/Standard Diluent Buffer: 20 ml
4. Biotin Detection Reagent (Dilution 1:100): 120 µl
5. Detection Reagent diluent buffer: 14 ml
6. HRP Conjugate Reagent (Dilution 1:100): 120 µl
7. HRP diluent buffer: 14 ml
8. TMB substrate: 10 ml
9. Stop solution: 10 ml
10. Wash buffer (25X): 30 ml
11. Plate Sealer: 5

Material Required But Not Provided

1. 37°C incubator
2. Microplate reader (wavelength: 450 nm)
3. Precision pipette and disposable pipette tips
4. Automated plate washer
5. ELISA shaker
6. 1.5 ml tubes to prepare standard/sample dilutions
7. Absorbent filter papers
8. 100 ml and 1 liter graduated cylinders

Protocol

A. Preparation of sample and reagents

1. Sample

Store samples to be assayed within 24 hours at 2-8°C. Alternatively, aliquot and store at -20°C or -80°C for long term. Avoid repeated freeze-thaw cycles.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 60 minutes. Centrifuge at approximately 1000 × g for 15 min. Analyze the serum immediately or aliquot and store at -20°C or -80°C.
- **Plasma:** Collect plasma using EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store at -20°C. Avoid hemolysis and high cholesterol samples.
- **Tissue homogenates:** The preparation of tissue homogenates will vary depending upon tissue type – this is just an example. Rinse tissues with ice-cold PBS to remove the excess of blood. Weigh before homogenization. Finely mince tissues and homogenize with a tissue homogenizer on ice in PBS and sonicate the cell suspension. Centrifuge the homogenates at 5000 × g for 5 min and collect the supernatant. Assay immediately or aliquot and store at -20°C.
- **Other biological fluids:** Centrifuge at approximately 1000 × g for 20 min to remove precipitant. Analyze immediately or aliquot and store at -20°C or -80°C.

Note:

» Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results. It is recommended to store samples to be used within 5 days at 4°C, within 1 month at -20°C and within 2 months at -80°C.

» Always use non-pyrogenic, endotoxin-free tubes for blood collection.

» Samples should be clear and transparent. Samples must be diluted so that the expected concentration falls within the kit's range.

» Please bring sample slowly to room temperature. Sample hemolysis will influence the result. Hemolyzed specimen should not be used. Samples that contain NaN_3 cannot be detected as it interferes with HRP.

» Always use non-pyrogenic, endotoxin-free tubes for blood collection.

Sample dilution guideline:

Estimate the concentration of the target in the sample and select the correct dilution factor to make the diluted target concentration fall near the middle of the kit's range. Generally, for high concentration (5000 ng/ml - 50000 ng/ml), dilute 1:100, for medium concentration (500 ng/ml - 5000 ng/ml), dilute 1:10 and for low concentration (7.813 ng/ml - 500 ng/ml), dilute 1:2. Very low concentrations (≤ 7.813 ng/ml) do not need dilution. Dilute the sample with the provided Sample Diluent Buffer and mix thoroughly. Several trials may be necessary to determine the optimal dilution factor.

2. Wash buffer

Dilute the concentrated Wash buffer 25-fold (1/25) with distilled water (i.e. add 30 ml of concentrated wash buffer into 720 ml of distilled water).

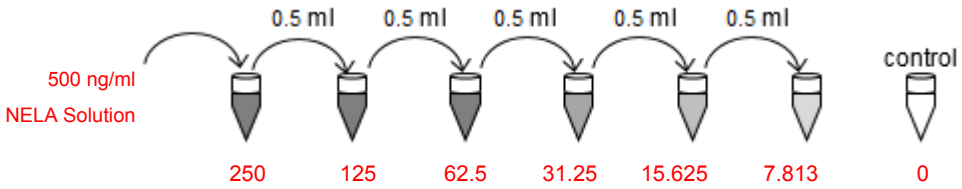
3. Standard

Preparation of the NELA standard: standard solution should be prepared no more than 15 min prior to the experiment. Centrifuge at 10,000×g for 1 minute as the powder may drop off from the cap when opening. **(Note: Do not dilute the standard directly in the plate).** Once your standard has been reconstituted, it should be used right away. We do not recommend reusing the reconstituted standard.

a.) 500 ng/ml standard solution. Add 1 ml of Sample/Standard diluent buffer into one Standard tube, keep the tube at room temperature for 10 min, mix gently (do not vortex as this will destabilize the protein) and avoid foaming or bubbles.

b.) 250 ng/ml → 7.8125 ng/ml standard solutions: Label 6 tubes with 250 ng/ml, 125 ng/ml, 62.5 ng/ml, 31.25 ng/ml, 15.625 ng/ml and 7.8125 ng/ml. Aliquot 0.5 ml of the Sample / Standard diluent buffer into each tube. Add 0.5 ml of the above 500 ng/ml standard solution into 1st tube and mix thoroughly. Transfer 0.5 ml from 1st tube to 2nd tube and mix thoroughly. Transfer 0.5 ml from 2nd tube

to 3rd tube and mix thoroughly, and so on.



4. Preparation of Biotin Detection Reagent working solution: prepare no more than 1 hour before the experiment.
a.) Calculate the total volume of the working solution: 0.1 ml / well × quantity of wells. (Allow 0.1-0.2 ml more than the total volume).
b.) Dilute the Biotin Detection Reagent with Detection Reagent diluent buffer at 1/100 and mix thoroughly. i.e. Add 1 µl of Biotin Detection Reagent into 99 µl of Detection Reagent diluent buffer. Discard after use.

5. Preparation of HRP Conjugated Reagent working solution: prepare no more than 30 min. before the experiment
a.) Calculate the total volume of the working solution: 0.1 ml / well × quantity of wells. (Allow 0.1-0.2 ml more than the total volume).
b.) Dilute the HRP Conjugate Reagent with HRP diluent buffer at 1/100 and mix thoroughly. i.e. Add 1 µl of HRP Conjugate Reagent into 99 µl of HRP diluent buffer. Discard after use.

B. Assay Procedure

1. Set standard, test sample and control (zero) wells on the pre-coated plate and record their positions. It is recommended to measure each standard and sample in duplicate.
2. Add 100 µl of the prepared standards solutions into the standard wells.
3. Add 100 µl of Sample / Standard diluent buffer into the control (zero) well.
4. Add 100 µl of appropriately diluted sample into test sample wells.
5. Cover the plate and incubate at 37°C for 90 minutes.
6. Remove the cover and discard the liquid. Do not wash.
7. Add 100 µl of prepared Biotin Detection Reagent working solution into each well (standard, test sample and zero well). Add the solution at the bottom of each well without touching the side walls. Seal the plate with a cover and incubate at 37°C for 60 minutes.
8. Remove the cover, and wash the plate 3 times. Discard the solution without touching the side walls. Blot the plate on an absorbent material. Fill each well completely with wash buffer and soak for at least 1-2 min. Discard the contents and clap the plate on absorbent filter papers or other absorbent material. Repeat this procedure for a total of three times.

Please note: For automated washing, discard the solution in all wells and wash three times overfilling the wells with Wash buffer. After the final wash invert the plate and tap on absorbent filter papers or other absorbent material. It is recommended that the washer be set for a soaking time of 1 min.

9. Add 100 µl of HRP working solution into each well, cover the plate and incubate at 37°C for 30 minutes.
10. Remove the cover and wash the plate 5 times with Wash buffer. Allow the wash buffer to remain in the wells 1-2 min for each wash. Discard the washing buffer and blot the plate onto absorbent filter papers or other absorbent material.
11. Add 90 µl of TMB substrate into each well. Cover the plate and incubate at 37°C in dark conditions for 15-20 minutes (incubation time is for reference only, do not exceed 30 minutes). When an apparent gradient appears in the standard wells the reaction can be terminated.

12. Add 50 µl of Stop solution into each well. There should be a color change to yellow. Gently tap the plate to ensure thorough mixing.
13. Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles. Measure the absorbance at 450 nm immediately.

For calculation, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of Zero well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). Log-log curve fitting is recommended for data analysis. The Human NELA concentration of the samples can be interpolated from the standard curve.

Note: If the samples measured were diluted, multiply the dilution factor by the concentrations from interpolation to obtain the concentration before dilution.

C. Precautions

1. Equilibrate all reagents to room temperature (18-25°C) prior to use and centrifuge the tubes briefly in case any contents are trapped in the lid.
2. For each step in the procedure, total dispensing time for addition of reagents to the assay plate should not exceed 10 minutes.
3. Wash buffer may crystallize and separate. If this happens, please warm the tube and mix gently to dissolve.
4. Avoid foaming or bubbles when mixing or reconstituting components. Prepare the Standard solutions within 15 min of starting the experiment. Please use the diluted Standard for a single assay procedure and discard after use.
5. It is recommended to assay all standards, controls and samples in duplicate or triplicate.
6. Do NOT let the plate dry out completely during the assay as this will inactivate the biological material on the plate.
7. Ensure plates are properly sealed or covered during incubation steps.
8. Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings.
9. To avoid cross contamination do not reuse pipette tips and tubes.
10. Do not use components from a different kit or expired ones.
11. The TMB Substrate solution is easily contaminated; work under sterile conditions when handling the TMB substrate solution. The TMB Substrate solution should also be protected from light. Unreacted substrate should be colorless or very light yellow in appearance. Aspirate the dosage needed with sterilized tips and do not dump the residual solution back into the vial.

D. Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, medium and high levels of NELA were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, medium and high levels of NELA were tested on 3 different plates, 8 replicates in each plate.

CV (%) = (Standard Deviation / mean) × 100

Intra-Assay: CV<10%

Inter-Assay: CV<10%

E. Typical Data & Standard Curve

Typical Standard Curve Data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

Concentration ng/ml	0	7.8125	15.625	31.25	62.5	125	250	500
OD450	0.066	0.119	0.169	0.247	0.495	0.988	1.676	2.394