ImmunoDiagnostics Limited

Human neutrophil elastase (NE) immunoassay kit

Catalogue Number: 31330

For the quantitative determination of human neutrophil elastase concentrations in serum and plasma samples.

This package insert must be read in its entirety before using this product.

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INTRODUCTION

Neutrophil elastase (NE), also known as leukocyte elastase, serine elastase, and elaszym, is one of the hematopoietic serine proteases localized in the primary granules of polymorphonuclear neutrophils (PMNs) [1]. The primary function of NE is recognized as to participate in the clearance of invaded pathogens through its intracellular and extracellular killing as well as antimicrobial activity, and the degradation of extracellular matrix components, including elastin, collagens, fibroncetin and proteoglycans [2]. Accumulated evidence has also demonstrated that NE can regulate inflammatory process through promoting chemokine and cytokine activation and degradation, cytokine receptor shedding, proteolysis of cytokine binding proteins and the activation of different specific cell surface receptors [3, 4]. The NE activity is strictly controlled by a set of associated endogenous inhibitors, such as the alpha-1 antitrypsin (A1AT) and elafin [5]. Once they escape from the exact regulation, neutrophil serine proteases become invasive and destructive within a human body thereby contributing to a wide range of pathologies, such as chronic obstructive pulmonary disease (COPD), cystic fibrosis, atherosclerosis, diabetes mellitus [6-9].

PRINCIPLE OF THE ASSAY

This assay is a quantitative sandwich ELISA. The immunoplate is pre-coated with a polyclonal antibody specific for human NE. Standards and samples are pipetted into the wells and any human NE present is bound by the immobilized antibody. After washing away any unbound substances, a horseradish peroxidase (HRP)-linked polyclonal antibody specific for human NE is added to the wells. After a final wash step, an HRP substrate solution is added and colour develops in proportion to the amount of human NE bound initially. The assay is stopped and the optical density of the wells determined using a microplate reader. Since the increases in absorbance are directly proportional to the amount of captured human NE, the unknown sample concentration can be interpolated from a reference curve included in each assay.

INTENDED USE

This human NE ELISA kit is designed for quantification of human NE in serum and plasma samples.

REAGENTS SUPPLIED

Each kit is sufficient for one 96-well plate and contains the following components:

1. Micro-titre Strips (96 wells)- Coated with a polyclonal antibody against human NE, sealed.
2. 10×Wash buffer- 40 ml.
3. 5×Assay buffer- 30 ml.
4. 100×Detection antibody solution- A HRP labelled polyclonal antibody against human NE, 0.12 ml.
5. Human NE standard-10 ng of native human NE in a buffered protein base, lyophilised.
6. Substrate solution- 12 ml, ready for use.
7. Stop solution- 12 ml, ready for use.

OTHER MATERIALS REQUIRED, BUT NOT PROVIDED

1. Pipettes and pipette tips.
2. 96-well plate or manual strip washer.
3. Buffer and reagent reservoirs.
4. Paper towels or absorbent paper.
5. Plate reader capable of reading absorbency at 450 nm.
6. Distilled water or deionized water.

STORAGE

The kit should be stored at 2-8°C upon receipt, and all reagents should be equilibrated to room temperature before use. Remove any unused antibody-coated strips from the human NE microplate, return them to the foil pouch and re-seal. Once opened, the strips may be stored at 2-8°C for up to one month.

PREPARATION OF REAGENTS

Bring all reagents and materials to room temperature before assay.

A. 1×Assay buffer.

Prepare 1×Assay buffer by mixing the 5×Assay buffer (30 ml) with 120 ml of distilled water or deionized water. If precipitates are observed in the 5× Assay buffer bottle, warm the bottle in a 37°C water bath until the precipitates disappear. The 1×Assay buffer may be stored at 2- 8°C for up to one month.

B. 1×Wash buffer.

Prepare 1×Wash buffer by mixing the 10×Wash buffer (40 ml) with 360 ml of distilled water or deionized water. If precipitates are observed in the 10× Wash buffer bottle, warm the bottle in a 37°C water bath until the precipitates disappear. The 1×Wash buffer may be stored at 2-8°C for up to one month.

C. 1×Detection antibody solution.

Spin down the 100×Detection antibody solution briefly and dilute the desired amount of the antibody 1:100 with 1×Assay buffer, 100 µl of the 1×Detection antibody solution is required per well. Prepare only as much 1×Detection antibody solution as needed. Return the 100×Detection antibody solution to 2-8°C immediately after the necessary volume is removed.

PREPARATION OF STANDRADS AND SAMPLE

Human NE standards: Reconstitute the lyophilised standard with 1 ml of 1×Assay buffer to generate a standard stock solution of 10 ng/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare serially diluted standards using 1×Assay buffer as follows:

Standard volume	Volume of 1×Assay buffer	Concentration
10 ng/ml stock	-	10 ng/ml
250 µl of 10 ng/ml	250 µl	5 ng/ml
250 µl of 5 ng/ml	250 µl	2.5 ng/ml
250 µl of 2.5 ng/ml	250 µl	1.25 ng/ml
250 µl of 1.25 ng/ml	250 µl	0.625 ng/ml
250 µl of 0.625ng/ml	250 µl	0.312ng/ml
250 µl of 0.312 ng/ml	250 µl	0.156 ng/ml

1×Assay buffer serves as the zero standard (0 ng/ml).

Note: The reconstituted standard stock should be aliquoted and stored at -80ºC for up to one month. Avoid repeating freezing/thawing cycles. Please do not store the diluted standard solutions.

Sample preparation

Serum or plasma sample is generally required a 100-fold dilution in the 1×Assay buffer. A suggested dilution step is to add 10 µl of sample to 990 µl of 1×Assay buffer. If a sample has a NE level greater than the highest standard, the sample should be diluted further and the assay should be repeated.

ASSAY PROCEDURE

It is recommended that all standards and samples should be assayed in duplicate.

1. Add 100 µl of standard or sample per well, incubate at room temperature for 1 hour.
2. Discard the content and tap the plate on a clean paper towel to remove residual solution in each well. Add 300 µl of 1×Wash buffer to each well and incubate for 1 minute. Discard the 1×Wash buffer and tap the plate on a clean paper towel to remove residual wash buffer. Repeat the wash step for a total 3 washes.
3. Add 100 µl of 1×Detection antibody solution to each well, incubate at room temperature for 1 hour.
4. Wash each well 4 times as described in step 2.
5. Add 100 µl of Substrate solution to each well, incubate at room temperature for 15 minutes. **Protect from light**.
6. Add 100 µl of Stop solution to each well, gently tap the plate frame for a few seconds to ensure thorough mixing.
7. Measure absorbance of each well at 450 nm immediately.

CALCULATION

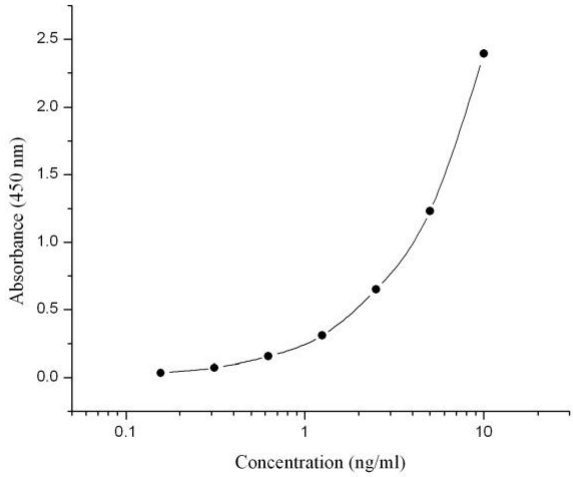
1. Subtract absorbance of blank from that of standards and samples.
2. Generate a standard curve by plotting the absorbance obtained (y-axis) against human NE concentrations (x-axis). The best fit line can be generated with any curve-fitting software by regression analysis. Any curve of 4-parameter or log-log curve fitting can be used for calculation.
3. Determine human NE concentration of samples from standard curve and multiply the value by the dilution factor.

TYPICAL STANDARD CURVE

The following standard curve is provided for demonstration only. A standard curve should be generated for each set of sample assay.

Human NE (ng/ml)	Absorbance (450 nm)	Blanked Absorbance
0	0.07	0
0.156	0.105	0.035
0.312	0.142	0.072
0.625	0.225	0.155
1.25	0.379	0.309
2.5	0.72	0.65
5	1.3	1.23
10	2.463	2.393

Human NE standard curve (4-parameter)



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SUMMARY OF ASSAY PROCEDURE

Add 100 µl of Standard or sample to each well.
↓
Incubate at room temperature for 1 hour.
↓
Aspirate and wash each well three times.
↓
Add 100 µl of 1×Detection antibody solution to each well.
↓
Incubate at room temperature for 1 hour.
↓
Aspirate and wash each well four times.
↓
Add 100 µl of Substrate solution to each well.
↓
Incubate at room temperature for 15 minutes.
↓
Add 100 µl of Stop solution to each well.
↓
Measure absorbance of each well at 450 nm.
↓
Calculation

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