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Neutrophil Elastase Activity Assay Kit (Fluorometric)

12/14

(Catalog # K383-100, 100 assays, Store kit at -20°C)

I. Introduction:

Neutrophil Elastase (NE, EC 3.4.21.37, leukocyte elastase, ELANE, ELA2, elastase 2, neutrophil elaszym, or serine elastase) is a cytotoxic serine protease. It is stored in the azurophil granules of neutrophil granulocyte and is released following cell stimulation, e.g. by pathogens, immune complexes or chemotactic agents (PMA). When the extracellular NE concentration exceeds the buffering capacity of endogenous inhibitors, it causes degradation of a wide range of extracellular matrix proteins, including fibronectin, laminin, proteoglycans, collagens, and elastin. Extracellular elastase is implicated in the signs, symptoms and disease progression of inflammatory lung disorders (such as cystic fibrosis, COPD, lung emphysema) via its role in the inflammatory processes, mucus overproduction and lung tissue damage. BioVision's Neutrophil Elastase Activity Assay Kit utilizes the ability of NE to proteolytically cleave a synthetic substrate and release a fluorophore, AFC, which can be easily quantified by fluorescence microplate readers. This assay kit is simple, rapid and can detect as low as 1 ng of Neutrophil Elastase in a variety of samples.

Neutrophil Elastase Substrate-AFC $\xrightarrow{\text{Neutrophil Elastase}}$ Cleaved substrate + AFC (Fluorescence)

II. Applications:

- Determine activity of purified Neutrophil Elastase
- Detect the activity of Neutrophil Elastase in plasma, blood
- Screen/study/characterize chemotactic agents causing Neutrophil stimulation and release of Elastase

III. Sample Type:

- Purified enzyme
- Blood, plasma

IV. Kit Contents:

Components	K383-100	Cap Code	Part Number
NE Assay Buffer	15 ml	WM	K383-100-1
NE Dilution Buffer	1 ml	Clear	K383-100-2
NE Substrate	0.2 ml	Red	K383-100-3
NE Enzyme Standard (1 µg)	1 vial	Green	K383-100-4

V. User Supplied Reagents and Equipment:

- 96-well white microplate with flat bottom
- Multi-well spectrophotometer.

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials at low speed prior to opening. Read entire protocol before performing the assay.

- **NE Assay Buffer:** Bring to room temperature before use.
- **NE Enzyme Standard:** Reconstitute with 10 µl of NE Dilution Buffer to prepare 100 ng/µl of stock solution. Mix well by pipetting up and down. Aliquot and store at -80°C. Avoid repeated freeze/thaw.

VII. Neutrophil Elastase Activity Assay Protocol:

1. **Sample Preparation:** Add 2-50 µl of purified enzyme or blood or plasma sample per well of 96-well plate and adjust the volume to 50 µl/well with NE Assay Buffer.

   Note:

   a) To study chemotactic agents causing Neutrophil stimulation and release of Elastase, we recommend treating blood with Red Blood Cell Lysis Buffer (Catalog # 5830) to isolate leukocytes prior to the treatment with a chemotactic agent.

   b) (Optional) for samples having fluorescence background, prepare in parallel sample background control well(s) containing sample only and adjust the volume to 100 µl with NE Assay Buffer.

2. **NE Standard Curve Preparation**: Prepare 5 ng/µl of NE Enzyme working solution by adding 38 µl of NE Assay Buffer to 2 µl of NE Enzyme stock solution (100 ng/µl). Add 0, 1, 2, 3, 4, and 5 µl of NE Enzyme working solution (5 ng/µl) into a series of wells in a 96-well plate to prepare 0, 5, 10, 15, 20 and 25 ng/well of NE Standard. Adjust the volume to 50 µl/well with NE Assay Buffer.

   Note: Store NE Enzyme Working Solution at -80°C and use within a week.

3. **NE Substrate Mix:** Prepare enough reagents for the number of assays to be performed. Prepare 50 µl of NE Substrate Mix for Standard and sample wells.

NE Assay Buffer	48 µl
NE Substrate	2 µl

   Mix and add 50 µl of NE Substrate Mix into Standard and sample well(s). Mix well.

4. **Measurement:** Measure fluorescence in kinetic mode for 10-20 min. at 37°C (Ex/Em = 380/500 nm). Choose two time points (T_1 and T_2) in the linear range of the plot and obtain the corresponding values for the fluorescence (RFU1 and RFU2).

5. **Calculations**: Subtract 0 Standard reading from all readings. Plot the Neutrophil Elastase Standard Curve. Apply sample's ΔRFU to Neutrophil Elastase Standard Curve to obtain corresponding Neutrophil Elastase (B, in ng) and calculate the activity of Neutrophil Elastase in the sample as:

$$\text{Sample Neutrophil Elastase Activity} = \frac{B}{V} \times Dilution\ Factor = \frac{ng}{ml} = \frac{\mu g}{L}$$

Where B is NE amount in the sample well from Standard Curve (ng)
V is sample volume added into the reaction well (ml)

Note: If the sample background control reading is significant, subtract the sample background control fluorescence reading from sample reading.

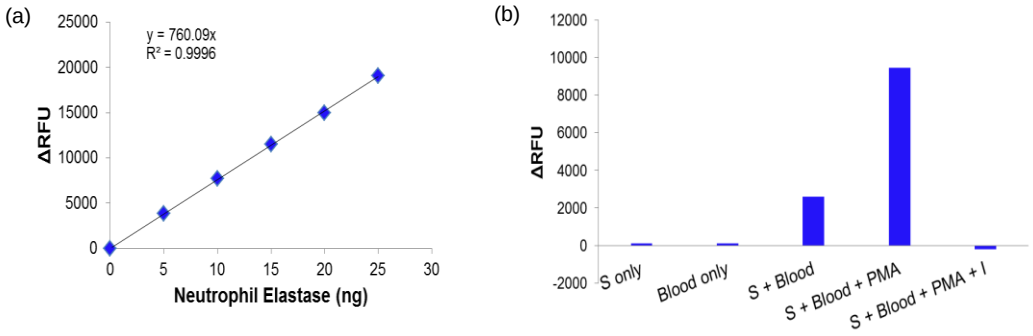

Figure: (a) Standard plot of Neutrophil Elastase activity. (b) Neutrophil Elastase activity was measured in human blood, following removal of RBCs and stimulation of neutrophils by 100 nM PMA (Catalog # 1544-5). S = Substrate, I = Inhibitor (60 µM SPCK). Assays were performed following the kit protocol.

VIII. RELATED PRODUCTS:

Elastase, Human Neutrophil (4716)
Phorbol-12-myristate 13-acetate (1544)
MMP-12 Antibody (3532)
Red Blood Cell Lysis Buffer (5830)
Neutrophil Elastase Inhibitor Screening Kit (Fluorometric) (K782)
Elastase Inhibitor, SPCK (1921)
Chymotrypsin, Human Pancreas (7538)

FOR RESEARCH USE ONLY! Not to be used on humans.