

# **ab204730**

## **Neutrophil Elastase Activity Assay Kit (Fluorometric)**

### Instructions for Use

For rapid, sensitive and accurate detection of Neutrophil Elastase activity.

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

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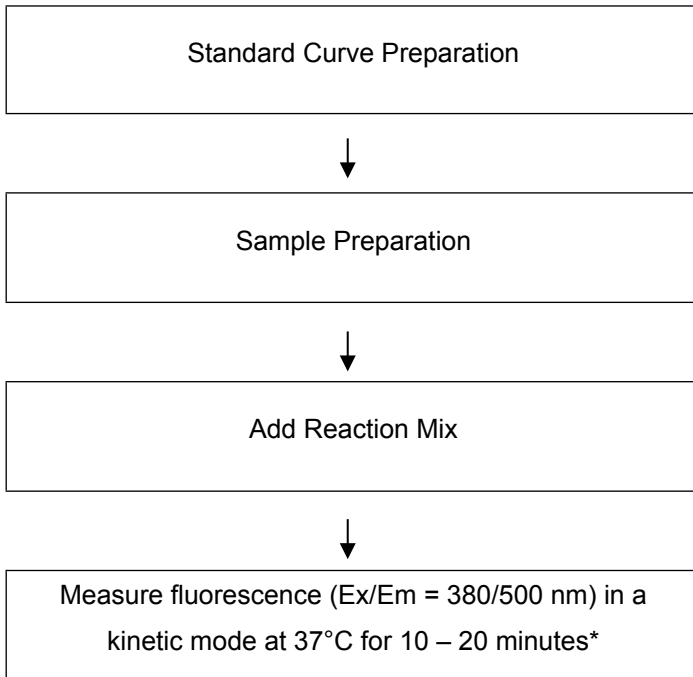
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## 1. BACKGROUND

Neutrophil Elastase Activity Assay Kit (Fluorometric) (ab204630) is based on the ability of neutrophil elastase (NE) to proteolytically cleave a synthetic substrate in order to 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 (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 disorder (such as cystic fibrosis, COPD, lung emphysema) via its role in the inflammatory processes, mucus overproduction and lung tissue damage.

## 2. ASSAY SUMMARY



*\*For kinetic mode detection, incubation time given in this summary is for guidance only.*

### 3. PRECAUTIONS

**Please read these instructions carefully prior to beginning the assay.**

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

### 4. STORAGE AND STABILITY

**Store kit at -20°C in the dark immediately upon receipt. 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 Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature. **Reconstituted components are stable for 2 months.**

### 5. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

## 6. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
NE Assay Buffer	15 mL	-20°C	-20°C
NE Dilution Buffer	1 mL	-20°C	-20°C
Substrate VI/NE Substrate	200 µL	-20°C	-20°C
Elastase Enzyme/NE Enzyme Standard	1 µg	-20°C	-80°C

## 7. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully perform this assay:

- Red Blood Cell (RBC) Lysis Buffer (ab204733) (if using blood)
- Pipettes and pipette tips
- Fluorescent microplate reader – equipped with filter Ex/Em = 380/500 nm
- 96 well plate with flat bottom preferably white
- Heat block or water bath

### 8. TECHNICAL HINTS

- **This kit is sold based on number of tests. A ‘test’ simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Keep enzymes, heat labile components and samples on ice during the assay.
- Make sure all buffers and solutions are at room temperature before starting the experiment.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Make sure you have the right type of plate for your detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on.

## 9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening.

### 9.1 **NE Assay Buffer:**

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

### 9.2 **NE Dilution Buffer:**

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

### 9.3 **Substrate VI/NE Substrate:**

Ready to use as supplied. Equilibrate to room temperature before use. Aliquot Substrate so that you have enough volume to perform the desired number of assays. Store at -20°C.

### 9.4 **Elastase Enzyme/NE Enzyme Standard:**

Reconstitute the Elastase Enzyme/NE Enzyme Standard with 10  $\mu\text{L}$  of NE Dilution Buffer to prepare 100 ng/ $\mu\text{L}$  standard stock solution. Aliquot standard so that you have enough volume to perform the desired number of assays. Store at -80°C. Avoid repeated freeze/thaw. Keep on ice while in use.



## 10. STANDARD PREPARATION

- Always prepare a fresh set of standards for every use.
  - Diluted standard solution is stable at  $-80^{\circ}\text{C}$  for up to one week.
- 10.1 Prepare a 5 ng/ $\mu\text{L}$  Elastase Enzyme/NE Enzyme Standard by diluting 4  $\mu\text{L}$  of the reconstituted Elastase Enzyme/NE Enzyme stock solution (100 ng/ $\mu\text{L}$ ) with 76  $\mu\text{L}$  of NE Assay Buffer.
- 10.2 Using 5 ng/ $\mu\text{L}$  Elastase Enzyme/NE Enzyme Standard, prepare standard curve dilution as described in the table in a microplate.

Standard #	Volume of Standard ( $\mu\text{L}$ )	Assay Buffer ( $\mu\text{L}$ )	Final volume standard in well ( $\mu\text{L}$ )	End Conc Elastase Enzyme/NE Enzyme in well (ng/well)
1	0	150	50	0
2	3	147	50	5
3	6	144	50	10
4	9	141	50	15
5	12	138	50	20
6	15	135	50	25

Each dilution has enough amount of standard to set up duplicate readings (2 x 50  $\mu\text{L}$ ).

## 11. SAMPLE PREPARATION

### **General Sample information:**

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step before storing the samples. Alternatively, if that is not possible, we suggest that you snap your sample in liquid nitrogen upon extraction and store the samples immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.

### 11.1 **Plasma and Blood:**

Plasma and Blood samples can be tested directly by adding sample to the microplate wells.

However, to find the optimal values and ensure your readings will fall within the standard values, we recommend performing several dilutions of the sample.

**NOTE:** *To study chemotactic agents causing neutrophil stimulation and release of elastase, we recommend treating blood with Red Blood Cell (RBC) Lysis Buffer (ab204733) to isolate leukocytes prior to the treatment with a chemotactic agent.*

## 12. ASSAY PROCEDURE and DETECTION

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- It is recommended to assay all standards, controls and samples in duplicate.
- Prepare all reagents, working standards, and samples as directed in the previous sections.

### 12.1 Set up Reaction wells:

12.1.1 Standard wells = 50  $\mu$ L standard dilutions.

12.1.2 Sample wells = 2 – 50  $\mu$ L samples (adjust volume to 50  $\mu$ L/well with NE Assay Buffer).

12.1.3 Background control sample wells = 2 - 50  $\mu$ L samples (adjust volume to 100  $\mu$ L/well NE Assay Buffer). **NOTE:** *for samples having fluorescence background.*

### 12.2 Reaction Mix:

Prepare 50  $\mu$ L of Reaction Mix for each reaction:

Component	Reaction Mix ( $\mu$ L)
NE Assay Buffer	48
Substrate VI/NE Substrate	2

Mix enough reagents for the number of assays (samples and controls) to be performed. Prepare a master mix of the Reaction Mix to ensure consistency. We recommend the following calculation: X  $\mu$ L component x (Number reactions +1).

- 12.3 Add 50  $\mu$ L of Reaction Mix into each standard and sample well. Do not add Reaction Mix to the background control wells. Mix well.
- 12.4 Measure output on a fluorescent microplate reader at Ex/Em = 380/500 nm in a kinetic mode, every 2 – 3 minutes, for 10 – 20 minutes at 37°C protected from light.

**NOTE:** Sample incubation time can vary depending on Neutrophil Elastase (NE) activity in the samples. We recommend measuring fluorescence in kinetic mode and then choosing two time points ( $T_1$  and  $T_2$ ) during the linear range.

RFU value at  $T_2$  should not exceed the highest RFU in the standard curve.

## 13. CALCULATIONS

- 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.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

13.1 Average the duplicate reading for each standard and sample.

13.2 If the sample background control is significant, then subtract the sample background control from sample reading.

13.3 Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.

13.4 Subtract standard curve absorbance at time 1 from absorbance at time 2 and plot this as a function of concentration of enzyme.

13.5 Draw the best smooth curve through these points to construct the standard curve. Calculate the trend line equation based on your standard curve data (use the equation that provides the most accurate fit).

13.6 Amount of Neutrophil Elastase is calculated as:

$$\Delta RFU_{380/500nm} = (RFU_2 - RFU_{2BG}) - (RFU_1 - RFU_{1BG})$$

Where:

RFU<sub>1</sub> is the sample reading at time T<sub>1</sub>.

RFU<sub>1BG</sub> is the background control sample at time T<sub>1</sub>.

RFU<sub>2</sub> is the sample reading at time T<sub>2</sub>.

RFU<sub>2BG</sub> is the background control sample at time T<sub>2</sub>.

13.7 Use the  $\Delta RFU_{380/500nm}$  to obtain B (in ng) of Neutrophil Elastase.

13.8 Activity of Neutrophil Elastase in the test is calculated as:

$$NE \text{ Activity} = \left(\frac{B}{V}\right) * D = ng/mL = \mu g/L$$

Where:

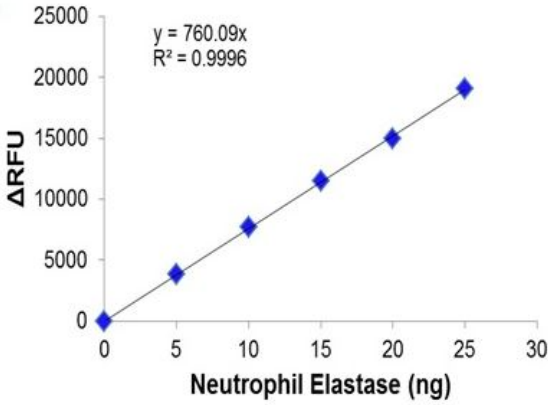
B = Amount of NE from Standard Curve (ng).

V = Original sample volume added into the reaction well (mL).

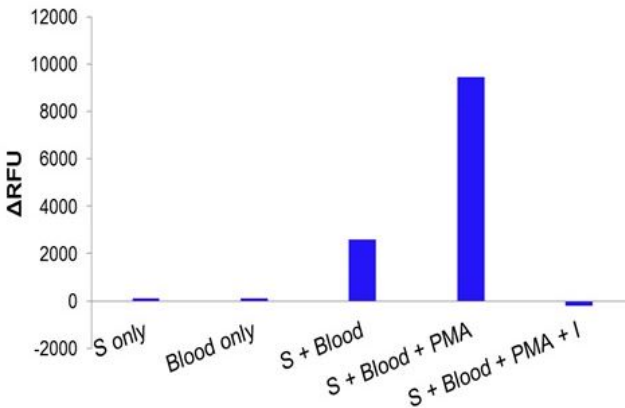
D = Sample dilution factor

14. TYPICAL DATA

**TYPICAL STANDARD CURVE** – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.



**Figure 1.** Typical Neutrophil Elastase Standard calibration curve using fluorometric reading.



**Figure 2.** Neutrophil Elastase activity was measured in human blood, following removal of RBCs and stimulation of neutrophils by 100 nM PMA. S = Substrate, I = Inhibitor (60 μM SPCK).

## 15. QUICK ASSAY PROCEDURE

**NOTE:** This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.

- Prepare standard and prepare enzyme mix; get equipment ready.
- Prepare appropriate standard curve.
- Prepare samples in duplicate (find optimal dilutions to fit standard curve readings).
- Set up plate for standard (50  $\mu$ L), samples (50  $\mu$ L) and background sample control (100  $\mu$ L) (optional) wells.
- Prepare Neutrophil Elastase Reaction Mix (Number samples + standards + 1).

Component	Reaction Mix ( $\mu$ L)
NE Assay Buffer	48
Substrate VI/NE Substrate	2

- Add 50  $\mu$ L of Neutrophil Elastase Reaction Mix to the standard and sample wells.
- Incubate plate at 37°C during 10-20 minutes and read fluorescence at Ex/Em= 380/500 nm in a kinetic mode.



## 16. TROUBLESHOOTING

<b>Problem</b>	<b>Cause</b>	<b>Solution</b>
Assay not working	Use of ice-cold buffer	Buffers must be at room temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different 96-well plate	Clear plates
Sample with erratic readings	Presence of interfering substance in the sample	Check protocol for interfering substances; deproteinize samples
Lower/ Higher readings in samples and Standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 $\mu$ L) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions on protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range

## 17. FAQ

### **Which chemicals or biological materials cause interference in this assay?**

RIPA buffer – contains SDS which can denature proteins and affect enzyme activity.

18. NOTES

## **Technical Support**

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