# Amplite® Fluorimetric Neutrophil Elastase Activity Assay Kit

Catalog number: 11327
Unit size: 100 Tests

| Component | Storage | Amount (Cat No. 11327) |
|---|---|---|
| Component A: NE Substrate | Freeze (< -15 °C), Minimize light exposure | 50 µL |
| Component B: NE Enzyme Standard | Freeze (< -15 °C) | 1 µg |
| Component C: NE Dilution Buffer | Freeze (< -15 °C) | 5 mL |
| Component D: NE Assay Buffer | Freeze (< -15 °C) | 5 mL |

## OVERVIEW

The Amplite® Fluorimetric Neutrophil Elastase Activity Assay Kit provides a robust and accurate method for quantifying neutrophil elastase (NE) activity. This assay is based on the proteolytic cleavage of a synthetic substrate by NE, which can be quantified at Ex/Em= 360/470 nm. It offers a direct measure of enzymatic activity proportional to the NE present in the samples. The kit's straightforward protocol is optimized for the sensitive detection of NE in blood and plasma samples and is suitable for screening and characterizing chemotactic agents triggering neutrophil stimulation. Neutrophil elastase, also known as leukocyte elastase, ELANE, ELA2, elastase 2, neutrophil, elaszym, serine elastase, subtype human leukocyte elastase (HLE) is a cytotoxic Serine Protease with a broad substrate specificity. Azurophil granules of neutrophil store NE and release them in response to multiple stimuli like pathogens, immune complexes, or chemotactic agents such as PMA causing degradation of a range of extracellular matrix proteins, including fibronectin, laminin, proteoglycans, collagens, and elastin. NE hydrolysis accounts for approximately 80% of the total protease hydrolysis activity in the human body. Disease implications of NE include cystic fibrosis, COPD, lung emphysema, rheumatoid arthritis, and adult respiratory distress syndrome. NE has also been associated with lung injury seen in COVID-19.

## AT A GLANCE

**Important Note**

Thaw all the kit components at room temperature before starting the experiment.

1. Prepare the test samples, and the serially diluted NE standards (50 µL).
2. Add the NE working solution (50 µL).
3. Incubate for 10-20 minutes at 37 °C.
4. Monitor the fluorescence intensity at Ex/Em=360/470 nm, cutoff=430 nm.

## KEY PARAMETERS

**Fluorescence microplate reader**

| | |
|---|---|
| Cutoff | 430 nm |
| Emission | 470 nm |
| Excitation | 360 nm |
| Recommended plate | Solid black |

## PREPARATION OF STOCK SOLUTIONS

*Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles*

**NE Standard Stock Soluton**

1. Add 10 µL of NE Dilution Buffer (Component C) to prepare a 100 µg/mL stock solution. Mix thoroughly by pipetting up and down.

   **Note:** Store the stock solution at -80 $^{0}$C, avoid repeated freeze/thaw cycles.

## PREPARATION OF STANDARD SOLUTIONS

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/11327

**NE Standard**
Add 3 µL of the 100 µg/mL NE standard solution to 297 µL of NE Dilution Buffer (Component C) to prepare a 1000 ng/mL NE solution (NE7). Then, take 100 µL of NE7 and perform 1:3 serial dilutions in NE Dilution Buffer (Component C) to create NE standards ranging from NE6 to NE1.

## PREPARATION OF WORKING SOLUTION

**NE Working Solution**

1. To prepare the NE working solution, add 50 µL of NE Substrate (100X) (Component A) to 5 mL of NE Assay Buffer (Component D) and mix thoroughly.

   **Note:** Prepare the NE working solution fresh before each experiment and protect it from light. A 5 mL solution is sufficient for 100 tests. Prepare the NE working solution in the required amount, adjusted proportionally to the number of tests needed.

## SAMPLE EXPERIMENTAL PROTOCOL

**Table 1.** Layout of NE standards and test samples in a 96-well solid black microplate. (STD = NE Standards (STD1-STD7, 4.1 to 1000 ng/mL), BL = Blank Control, TS = Test Samples)

| BL | BL | TS | TS |
|---|---|---|---|
| STD 1 | STD 1 | ... | ... |
| STD 2 | STD 2 | ... | ... |
| STD 3 | STD 3 | | |
| STD 4 | STD 4 | | |
| STD 5 | STD 5 | | |
| STD 6 | STD 6 | | |
| STD 7 | STD 7 | | |

**Table 2.** Reagent composition for each well.

| Well | Volume | Reagent |
|---|---|---|
| STD 1- STD 7 | 50 µL | Serial Dilutions (4.1 to 1000 ng/mL) |
| BL | 50 µL | NE Dilution Buffer (Component C) |
| TS | 50 µL | Test Sample |

1. Prepare the NE standards (STD1-STD7), blank controls (BL), and test samples (TS) as outlined in Tables 1 and 2. When using a 384-well plate, add 25 µL of reagent per well instead of 50 µL.

2. Add 50 µL of NE Working Solution to each well for NE standards, blank controls, and test samples. For a 384-well plate, add 25 µL of NE Working Solution to each well.

3. Incubate for 10-20 minutes at 37 °C, protected from light.

4. Monitor the fluorescence intensity with a fluorescence microplate reader at Ex/Em= 360/470 nm (Cutoff = 430 nm).

## EXAMPLE DATA ANALYSIS AND FIGURES

The fluorescence reading in the blank wells (containing only the assay buffer) serves as a control. These readings are subtracted from the values obtained in wells containing NE standards and test samples. The standard curve for NE is shown in Figure 1. To calculate the NE concentrations in the samples using this standard curve, we recommend using the Online Linear Regression Calculator available at:

https://www.aatbio.com/tools/linear-logarithmic-semi-log-regression-online-calculator

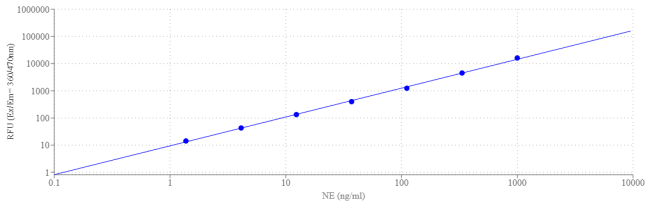


**Figure 1.** The dose response of neutrophil elastase (NE) was measured using the Amplite® Fluorimetric Neutrophil Elastase Assay Kit. The assay was conducted on a 96-well solid black microplate, utilizing a Gemini microplate reader (Molecular Devices) with Ex/Em = 360/470 nm, Cutoff = 430 nm.

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