Screen Quest™ Fluorimetric Neutrophil Elastase Inhibitor Screening Kit

Catalog number: 11802
Unit size: 100 Tests

Component	Storage	Amount (Cat No. 11802)
Component A: NE Substrate (100X)	Freeze (< -15 °C), Minimize light exposure	1 Vial (50 µL)
Component B: NE Enzyme	Freeze (< -15 °C)	1 Vial (5 µg)
Component C: NE Inhibitor (10 mM)	Freeze (< -15 °C)	1 Vial (25 µL)
Component D: NE Assay Buffer	Freeze (< -15 °C)	1 Bottle (15 mL)

OVERVIEW

The Screen Quest™ Fluorimetric Neutrophil Elastase Inhibitor Screening Kit offers a fast, sensitive, and high-throughput method for screening and characterizing potential inhibitors of NE. This assay measures the NE activity based on hydrolysis of the substrate, producing a fluorescent product with Ex/Em=360/470nm. The amount of fluorescent product is directly proportional to the enzymatic activity of the neutrophil elastase present in the sample. 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

Protocol Summary

1. Prepare the NE enzyme solution (40 µL/well).
2. Prepare the NE inhibitor serial dilution, and add 10 µL/well.
3. Incubate the NE enzyme with inhibitor for 5-10 minutes at 37 °C.
4. Add 50 µL of the NE working solution.
5. Incubate at room temperature for 10-30 minutes.
6. Monitor fluorescence intensity at Ex/Em = 360/470 nm, Cutoff = 430 nm.

Important Note

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

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 Enzyme Stock Solution

1. Add 50 µL of the NE Assay Buffer (Component D) to the vial of NE Enzyme (Component B) to make a 100 µg/mL NE Enzyme stock solution. Mix well by pipetting, aliquot, and store at -80 °C.

   Note: Must be used within 1 week of reconstitution. Avoid freeze/thaw cycles.

PREPARATION OF STANDARD SOLUTIONS

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/11802

NE Inhibitor Standard

Add 1.6 µL of NE Inhibitor (Component C) to 200 µL of NE Assay Buffer (Component D) to create an 80 µM NE Inhibitor solution (STD7). Take 100 µL of the STD7 solution and perform 1:2 serial dilutions using NE Assay Buffer (Component D). This process will produce a series of NE Inhibitor standards from STD7 to STD1.

PREPARATION OF WORKING SOLUTION

NE Enzyme Working Solution

1. Take 5 µL of the 100 µg/mL NE Enzyme stock solution and add it to 495 µL of NE Assay Buffer (Component D) to create a 1 µg/mL NE Enzyme solution.

   Note: The concentration of the NE Enzyme solution needs to be optimized when using a different inhibitor.

NE Substrate Working Solution

1. Add 25 µL of NE Substrate (100X) (Component A) to 5 mL of NE Assay Buffer (Component D), and mix well.

   Note: Prepare the NE working solution fresh before each experiment, and protect it from light.

   Note: A 5 mL solution is sufficient for 100 tests. Adjust the amount of NE working solution proportionally to match the number of tests you need.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of NE inhibitor serial dilution and test samples in a 96-well solid black microplate. (STD = NE Inhibitor Standards (STD1-STD7, 1.25-80 µM), BL= Blank Control, TS = Test Samples.)

Positive Control	Positive Control	TS	TS

STD 1	STD 1	...	...
STD 2	STD 2	...	...
STD 3	STD 3	BL	BL
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 (Total 50 µL)
STD 1-STD 7	40 µL NE Enzyme Solution + 10 µL NE Inhibitor Serial Dilution
BL	40 µL NE Enzyme Solution + 10 µL NE Assay Buffer
GGT Positive Control	50 µL NE Assay Buffer
TS	40 µL NE Enzyme Solution + 10 µL of Inhibitor Test Sample

1. Prepare NE Inhibitor standards (STD1-7), blank controls (BL), positive control, and test samples (TS) as outlined in Tables 1 and 2. For a 384-well plate, use 25 µL of reagent per well instead of 50 µL.

2. Incubate the NE enzyme with inhibitor for 5-10 minutes at 37 ^{0}C.

3. Add 50 µL of NE Substrate Working Solution to each well containing the NE Inhibitor standards (STD1-STD7), blank control, positive control, and test samples. For a 384-well plate, add 25 µL of GGT Working Solution to each well instead.

4. Incubate for 10-30 minutes at 37 °C, protected from light.

5. 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 from the blank wells (containing assay buffer only) is used as a control and subtracted from the readings of wells containing NE Inhibitor standards and test samples. Figure 1 shows the standard curve for the NE Inhibitor. To determine the NE concentrations in your 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

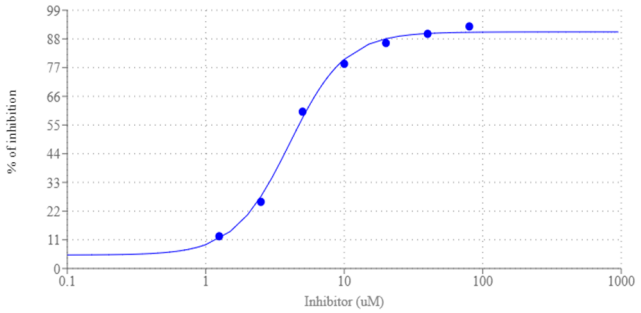

Figure 1. The NE Inhibitor dose response was measured using the Screen Quest™ Fluorimetric Neutrophil Elastase Inhibitor Screening Kit on a 96-well solid black microplate with a Gemini microplate reader (Molecular Devices) Ex/Em = 360/470 nm (Cutoff = 430 nm).

DISCLAIMER

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