EnzyFluo™ Elastase Inhibitor Assay Kit (EELI-100)

Fluorometric Inhibitor Screening Assay for Elastase

DESCRIPTION

PANCREATIC ELASTASE (PE, EC 3.4.21.36) and *NEUTROPHIL ELASTASE (NE, EC 3.4.21.37)* are serine proteases that exhibit similar elastin-degrading functions in different physiological roles. Neutrophil elastase is produced as an immune response to inflammation or infection, while pancreatic elastase hydrolyzes dietary proteins in many eukaryotic and prokaryotic organisms. BioAssay Systems' EnzyFluo™ Elastase Inhibitor Assay Kit uses a fluorescein isothiocyanate (FITC)-labeled synthetic substrate to quantify elastase activity in serine proteases. The fluorescein label is highly quenched. Upon digestion by elastase present in the sample, the substrate is cleaved into smaller peptides, which abolishes the quenching of the fluorescence label. The fluorescence or fluorescence polarization (FP) of the FITC-labeled fragments is measured at $\lambda_{ex/em}$ = 485/530 nm. Inhibition is determined by the decrease in fluorescence.

KEY FEATURES

Safe. Non-radioactive assay.

Homogeneous and convenient. Homogenous "mix-incubate-measure" type assay. No wash and reagent transfer steps are involved.

Robust and high-throughput. Can be readily automated to assay thousands of samples per day. Robust assay with a Z' factor of > 0.8.

APPLICATIONS

For evaluation of drugs and screening of potential inhibitors to elastase proteases.

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Assay Buffer: 12 mL **Inhibitor:** 100 µL
Substrate: 100 µL **Elastase:** 100 µL

Storage conditions. The kit is shipped on ice. Store all components at -20°C upon receipt. Shelf life: 6 months after receipt.

Precautions: Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to the Material Safety Data Sheet for detailed information.

PROCEDURES

Prior to the assay, equilibrate all components to room temperature. Elastase (human neutrophil elastase) should be kept on ice throughout the assay. This assay is based on a kinetic reaction. To ensure identical incubation time, addition of the Working Reagent to samples should be quick and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended. Assays can be executed at room temperature.

Sample Preparation:

Dissolve test compounds in a solvent of choice (e.g. DMSO). If using a different enzyme than the one provided in the kit, it is prudent to first test the tolerance of the solvent by the enzyme of choice. For human neutrophil elastase, the final DMSO concentration in the assay should be < 8%.

Inhibitor Screening in 96-well plate:

1. Dilute Elastase to 15 µg/mL by mixing 1 µL Elastase and 32 µL Assay Buffer per well. Transfer 30 µL of dilute Elastase into separate wells of a black, flat-bottom 96-well plate. Also, prepare 30 µL of elastase ("Control") and 30 µL of Assay Buffer ("Blank") wells, respectively.
2. To the Control and Blank wells, add 10 µL of the solvent in buffer that the test compounds are dissolved in. For example, if the test compounds are dissolved in buffer containing 0.1% DMSO, add 10 µL of this solution to these wells.
3. To the remainder of the wells containing enzyme, add 10 µL of the test compounds. Tap plate to mix and incubate for 15 min at room temperature to allow the inhibitor to block enzyme activity.
4. Prepare enough Working Reagent for all wells by mixing 1 µL Substrate and 84 µL Assay Buffer for each well. Add 80 µL of the Working Reagent to all wells. Briefly tap to mix. Incubate for 30 min at room temperature, protected from light. Read the fluorescence or FP at $\lambda_{ex/em}$ = 485/530 nm.

CALCULATION

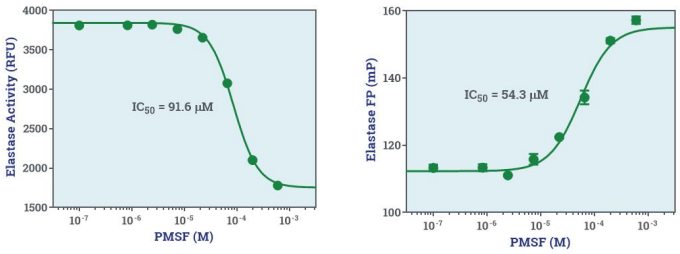
Elastase inhibition for a test compound is calculated as follows:

$$\text{Enzyme Activity \%} = \frac{(F_{Compound} - F_{Blank})}{(F_{Control} - F_{Blank})} \times 100\%$$

Where $F_{Compound}$, $F_{Control}$, and F_{Blank} are the fluorescence values at $\lambda_{ex/em}$ = 485/530 nm of the test compound, no inhibitor "Control", and the no enzyme "Blank" wells, respectively.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices and accessories (e.g. multi-channel pipettor), black, flat-bottom 96-well plates (e.g. Corning Costar), centrifuge tubes, and a plate reader capable of reading fluorescence or FP at $\lambda_{ex/em}$ = 485/530 nm.



Elastase inhibition curve. *Left:* Human Neutrophil Elastase was incubated with various concentrations of PMSF to generate a fluorescent IC_{50} curve. *Right*: A second IC_{50} curve was generated for FP measurements.

LITERATURE

1. Zeng, W., Song, Y., Wang, R., He, R., & Wang, T. (2023). Neutrophil elastase: From mechanisms to therapeutic potential. *Journal of pharmaceutical analysis*, *13*(4): 355–366.

2. James, H. L., & Cohen, A. B. (1978). Mechanism of inhibition of porcine elastase by human alpha-1-antitrypsin. *The Journal of clinical investigation*, 62(6): 1344–1353.

3. López-Otín, C., Bond, J. S. (2008). Proteases: multifunctional enzymes in life and disease. *Journal of Biological Chemistry*, 283(45): 30433–30437.