Description

The Neutrophil Elastase Inhibitor Screening Assay Kit is a fluorogenic assay designed to measure the activity of elastase for screening and profiling applications. The elastase assay kit comes in a convenient 96-well format and contains enough purified elastase, fluorochrome-conjugated substrate, and PR-02 buffer for 100 reactions. This kit also includes Sivelestat as control inhibitor.

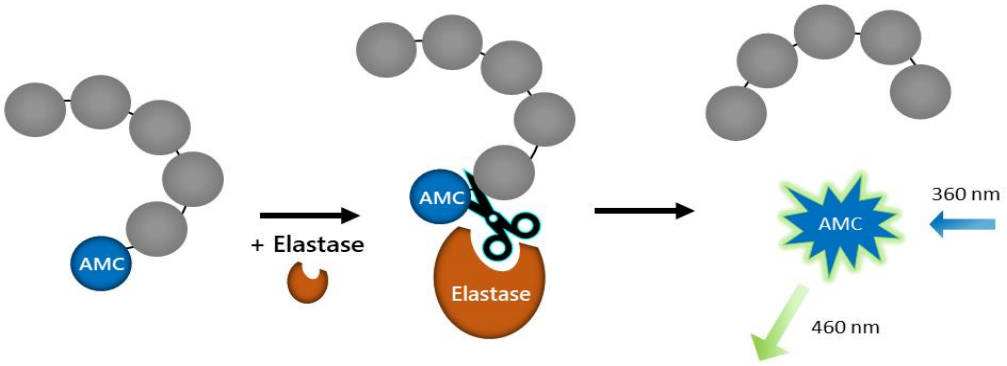

Figure 1: Illustration of the assay principle.

PR Substrate 3 is a fluorogenic peptide substrate for neutrophil elastase. In the conjugated form the energy emitted from the fluorochrome AMP is quenched. Proteolysis releases AMP and fluorescence is emitted. The increase in fluorescence is directly proportional to elastase activity.

Background

Elastase is a serine protease from the chymotrypsin family involved in the degradation of elastin and some viral proteins. Elastase exists as pancreatic and neutrophil elastase, which differ in their specificity. Neutrophil elastase is also known as ELA2 or polymorphonuclear leukocyte elastase and is present in azurophilic granules. Upon neutrophil activation, degranulation occurs and serine proteases, such as elastase, are released into the extracellular space to degrade the matrix proteins of pathogens. Alterations in the levels of elastase, or dysfunction of its endogenous inhibitors, result in alpha-1 antitrypsin deficiency (A1AD), emphysema, cyclic neutropenia, abdominal aortic aneurysms (AAA) and chronic obstructive pulmonary disease (COPD), arthritis and psoriasis. The use of neutrophil elastase inhibitors, such as elafin, and the development of new inhibitors with fewer side effects may benefit patients suffering from elastase-related diseases.

Applications

Screen small molecule inhibitors in high throughput screening (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
	Native Human Neutrophil Elastase*	> 1 µg	-80°C
	PR Substrate 3	6.25 µl	-80°C
	PR-02 Buffer	10 ml	-20°C
	10 mM Sivelestat	20 µl	-80°C
79685	96-well black microplate	1	Room Temp

** The concentration of protein is lot-specific and will be indicated on the tube containing the protein.*

Materials Required but Not Supplied

- Fluorescent microplate reader capable of reading lexcitation = 360 nm; lemission = 460 nm
- Adjustable micropipettor and sterile tips
- 30°C incubator

Stability

This assay kit will perform optimally for up to **6 months** from date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Assay Protocol

- All samples and controls should be performed in duplicate.
- The assay should include "Negative Control", "Positive Control", "Inhibitor Control" and "Test Inhibitor" conditions.
- If the assay plate is going to be used more than once, prepare enough reagents for this portion of the assay and aliquot the remaining undiluted reagents into single-use aliquots depending on how many times the assay plate will be used. Store the aliquots at -80°C or at -20°C as appropriate.

1. Thaw **Elastase** on ice. Briefly spin the tube to recover the full content.

2. Dilute Elastase to 0.25 ng/µl in **PR-02 Buffer** (20 µl/well).

   Note: Keep the diluted protein on ice until use. Discard any unused diluted protein after use.

3. Prepare the Test Inhibitor (5 µl/well): for a titration prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is 50 µl.

   3.1 If the Test Inhibitor is water-soluble, prepare 10-fold more concentrated serial dilutions of the inhibitor than the desired final concentrations in PR-02 Buffer.

   For the positive and negative controls, use PR-02 Buffer (Diluent Solution).

   OR

   3.2 If the Test inhibitor is soluble in DMSO, prepare the test inhibitor at a concentration 100-fold higher than the highest desired concentration in 100% DMSO, then dilute the inhibitor 10-fold in PR-02 Buffer to prepare the highest concentration of the 10-fold intermediate dilutions. The concentration of DMSO is now 10%.

   Using PR-02 Buffer containing 10% DMSO to keep the concentration of DMSO constant, prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations.

For positive and negative controls, prepare 10% DMSO in PR-02 Buffer (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

Note: The final concentration of DMSO should not exceed 1%.

4. Add 20 µl of diluted elastase to all wells except "Negative Control" wells.

5. Add 5 µl of test inhibitor to each well designated "Test Inhibitor".

6. Dilute 10 mM Sivelestat 10-fold with PR-02 Buffer (makes 1 mM solution in 10% DMSO). Further dilute 1 mM Sivelestat 100-fold with PR-02 Buffer containing 10% DMSO.

7. Add 5 µl of 10 µM Sivelestat to the "Control Inhibitor" wells.

8. Add 5 µl of Diluent Solution to the "Positive Control" and "Negative Control" wells.

9. Preincubate the "Test Inhibitor" and "Control Inhibitor" with the diluted Elastase for 30 minutes at Room Temperature (RT) with gentle agitation.

10. Dilute 400-fold the PR Substrate 3 in PR-02 Buffer.

11. Add 25 µl of the diluted substrate to all wells.

12. Protect your samples from direct exposure to light and incubate at RT for 30 minutes or perform kinetic analysis.

13. Read fluorescence intensity of the samples (lexcitation = 360 nm; lemission = 460 nm) in a fluorescence plate reader.

14. The blank should be subtracted from all other readings.

Component	**Negative Control**	**Positive Control**	**Control Inhibitor**	**Test Inhibitor**
PR-02 Buffer	20 µl	-	-	-
Test Inhibitor	-	-	-	5 µl
Diluted Sivelestat (10 µM)	-	-	5 µl	-
Diluent Solution	5 µl	5 µl	-	-
Diluted Elastase (0.25 ng/µl)	-	20 µl	20 µl	20 µl
30 minutes at Room Temperature				
Diluted PR Substrate 3 (400-fold diluted)	25 µl	25 µl	25 µl	25 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

Example Results

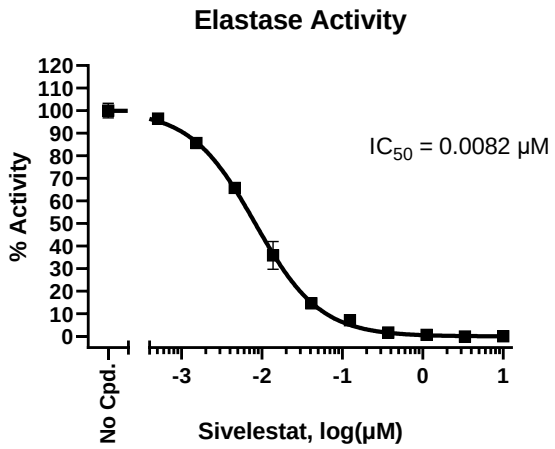

Figure 2. Elastase inhibition by Sivelestat.
Elastase activity was measured in the presence of increasing concentrations of Sivelestat. Results are expressed as percent activity, in which the activity of elastase in the absence of inhibitor is set to 100%.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com

Troubleshooting Guide
Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com

References
Jakimiuk K*, et al.*, 2021 *J Enzyme Inhib Med Chem* 36(1): 1016-1028.

Related Products

Products	*Catalog #*	*Size*
ELANE (Elastase), Avi-His-Tag HiP™ Recombinant	101141	25 µg / 100 µg
ELANE (Elastase), Avi-His-Tag, Biotin-Labeled HiP™ Recombinant	101142	25 µg / 50 µg