

Neutrophil Elastase Inhibitor Screening Kit (Fluorometric)

(Catalog #K782-100; 100 assays; Store kit at -20 °C)

I. Introduction:

Neutrophil Elastase (NE) is an aggressive and cytotoxic 29 kDa serine protease stored mainly in the azurophil granules of neutrophil granulocytes. It plays a role in the degradation of a wide range of extracellular matrix proteins, including fibronectin, laminin, proteoglycans, collagens, and elastin. When the extracellular NE concentration exceeds the buffering capacity of endogenous inhibitors, it becomes implicated in the signs, symptoms and disease progression of inflammatory lung disorders via its role in the inflammatory process, mucus overproduction and lung tissue damage. In BioVision's Neutrophil Elastase Inhibitor Screening Kit, NE hydrolyzes a specific fluorescent substrate to release the fluorescent group, which can be detected at Ex/Em = 400/505 nm. In presence of a potent Neutrophil Elastase inhibitor, the hydrolyzation of substrate will be inhibited or stopped. The kit provides a rapid, simple, sensitive, and reliable test suitable as a high throughput screening assay of Neutrophil Elastase inhibitors. For comparison of the relative efficacy of test inhibitors, a control inhibitor, SPCK ($K_i = 10 \mu\text{M}$ for human leukocyte elastase) is included.

II. Kit Contents:

Components	100 assays	Cap Color	Part Number
Assay Buffer	25 ml	WM	K782-100-1
Substrate	0.2 ml	Red	K782-100-2
Neutrophil Elastase	1 vial	Green	K782-100-3
Inhibitor Control (3 mM, SPCK)	100 μl	Purple	K782-100-4

III. Storage and Handling:

Store the kit at -20°C, protected from light. Allow Assay Buffer to warm to room temperature before use. Briefly centrifuge vials before opening. Read the entire protocol before performing the assay.

IV. Reagent preparation:

Neutrophil Elastase: Reconstitute the Neutrophil Elastase into 220 μl assay buffer. Aliquot and store the NE stock solution at -80°C. Avoid repeated freeze/thaw cycles. Use within one week.

V. Neutrophil Elastase Inhibitor Screening Assay Protocol:

1. Enzyme Preparation:

For each well, prepare a total 50 μl Neutrophil Elastase solution:
 48 μl Assay Buffer
 2 μl NE stock solution

2. Screen compounds, Inhibitor Control and Enzyme Control preparations:

Dissolve candidate compounds into a proper solvent. Dilute to 4X the final desired test concentration with Assay Buffer. For Inhibitor Control, dilute Inhibitor Control Stock 1:25 with Assay Buffer. Add 25 μl diluted test compounds, Inhibitor Control or Assay Buffer into NE enzyme wells as test inhibitors, Inhibitor Control, or Enzyme Control. Mix well, and incubate for 5 min at 37 °C.

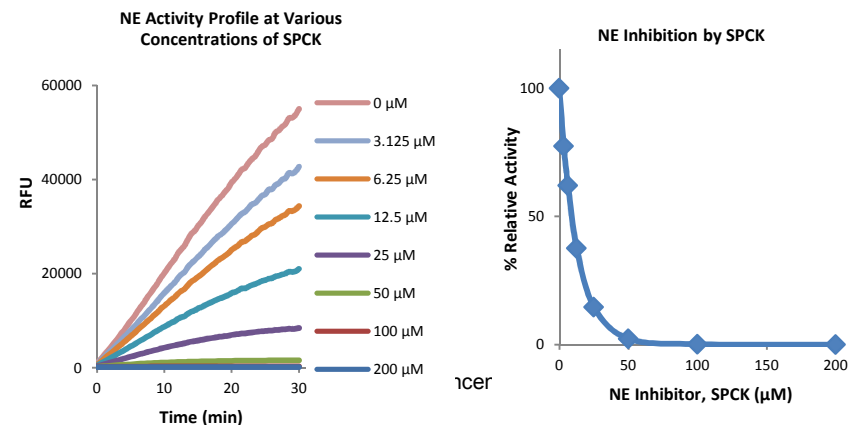
3. Reaction Mix: Mix enough reagents for the number of assays to be performed. For each well, prepare a total 25 μl Reaction Mix:

23 μl Assay Buffer
 2 μl Substrate

Add 25 μl of the Reaction Mix into each reaction well, mix, measure immediately.

4. Measurement: Read Ex/Em = 400/505 nm R_1 at T_1 . Read R_2 again at T_2 after incubating the reaction at 37°C for 30 min, protect from light. The RFU of fluorescence generated by hydrolyzation of substrate is $\Delta\text{RFU} = R_2 - R_1$. It is recommended to read kinetically to choose the R_1 and R_2 at linear range. Set the ΔRFU of Blank Control as the 100 % Relative Activity Value and calculate the relative activity for each candidate inhibitor as follows:

$$\% \text{ Relative Activity} = \frac{\Delta\text{RFU of candidate}}{\Delta\text{RFU of Enzyme Control}} \times 100 \%$$



RELATED PRODUCTS:

- MMP-1, 2, 3, 8, 9,13 human recombinant proteins
- MMP-1, 2, 3, 8, 9,11,12,13, 17, 19 Antibodies
- MMP-1 Inhibitor Screening Kit
- MMP-3 Inhibitor Screening Kit
- MMP-3 Activity Assay Kit
- MMP FRET Substrate
- GM6001
- Elastase Inhibitor, SPCK

FOR RESEARCH USE ONLY! Not to be used on humans.

GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	<ul style="list-style-type: none"> • Use of ice-cold assay buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate 	<ul style="list-style-type: none"> • Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	<ul style="list-style-type: none"> • Use of an incompatible sample type • Samples prepared in a different buffer • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples 	<ul style="list-style-type: none"> • Refer data sheet for details about incompatible samples • Use the assay buffer provided in the kit or refer data sheet for instructions • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed • Use fresh samples or store at correct temperatures until use
Lower/ Higher readings in Samples and Standards	<ul style="list-style-type: none"> • Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used 	<ul style="list-style-type: none"> • Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer datasheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	<ul style="list-style-type: none"> • Use of partially thawed components • Pipetting errors in the standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots 	<ul style="list-style-type: none"> • Thaw and resuspend all components before preparing the reaction mix • Avoid pipetting small volumes • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	<ul style="list-style-type: none"> • Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range 	<ul style="list-style-type: none"> • Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • Concentrate/ Dilute sample so as to be in the linear range
<p>Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.</p>		