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 µM 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.

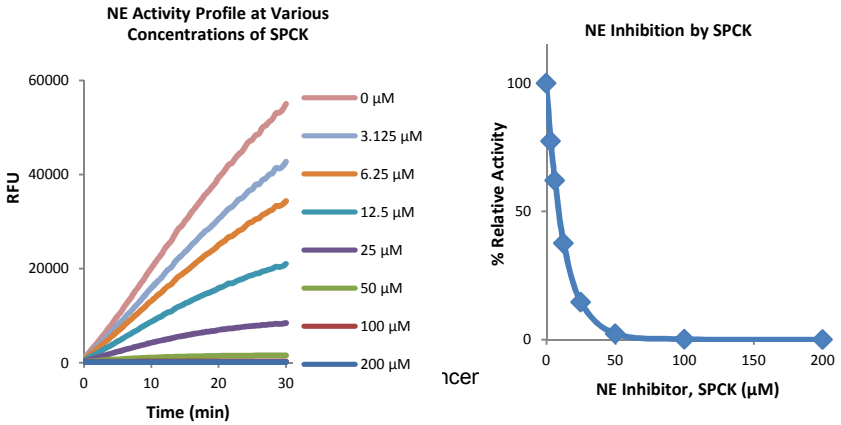
IV. Reagent preparation:

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 $\Delta RFU = R_2 - R_1$. 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:

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



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	• Use of ice-cold assay buffer	• Assay buffer must be at room temperature
	• Omission of a step in the protocol	• Refer and follow the data sheet precisely
	• Plate read at incorrect wavelength	• Check the wavelength in the data sheet and the filter settings of the instrument
	• Use of a different 96-well plate	• Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type	• Refer data sheet for details about incompatible samples
	• Samples prepared in a different buffer	• Use the assay buffer provided in the kit or refer data sheet for instructions
	• Cell/ tissue samples were not completely homogenized	• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope
	• Samples used after multiple free-thaw cycles	• Aliquot and freeze samples if needed to use multiple times
	• Presence of interfering substance in the sample	• Troubleshoot if needed
	• Use of old or inappropriately stored samples	• Use fresh samples or store at correct temperatures until use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components	• Thaw all components completely and mix gently before use
	• Use of expired kit or improperly stored reagents	• Always check the expiry date and store the components appropriately
	• Allowing the reagents to sit for extended times on ice	• Always thaw and prepare fresh reaction mix before use
	• Incorrect incubation times or temperatures	• Refer datasheet & verify correct incubation times and temperatures
	• Incorrect volumes used	• Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components	• Thaw and resuspend all components before preparing the reaction mix
	• Pipetting errors in the standard	• Avoid pipetting small volumes
	• Pipetting errors in the reaction mix	• Prepare a master reaction mix whenever possible
	• Air bubbles formed in well	• Pipette gently against the wall of the tubes
	• Standard stock is at an incorrect concentration	• Always refer the dilutions in the data sheet
	• Calculation errors	• Recheck calculations after referring the data sheet
	• Substituting reagents from older kits/ lots	• Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength	• Check the equipment and the filter setting
	• Samples contain interfering substances	• Troubleshoot if it interferes with the kit
	• Use of incompatible sample type	• Refer data sheet to check if sample is compatible with the kit or optimization is needed
	• Sample readings above/below the linear range	• Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		