Technical Bulletin

Neutrophil Elastase Inhibitor Screening Kit

Catalog Number MAK213

Product Description

Neutrophil elastase (NE) is a serine protease that hydrolyzes proteins within the azurophilic granules of neutrophils. Once secreted, it digests collagen-VI and elastin of the extracellular matrix. Neutrophil elastase is implicated in cystic fibrosis, bronchiectasis, chronic obstructive pulmonary disease, congenital neutropenia, and lung cancer.

The Neutrophil Elastase Inhibitor Screening Kit is a rapid, sensitive, and high throughput assay to screen and characterize potential inhibitors of NE. NE activity is measured by hydrolyzing the substrate to yield a fluorescent product (λ_{Ex} = 400 nm/ λ_{Em} = 505 nm) proportional to the enzymatic activity present.

The kit is suitable for the screening of inhibitors of Neutrophil Elastase.

Components

The kit is sufficient for 100 fluorometric assays in 96-well plates.

- Assay Buffer — 25 mL
  Catalog Number MAK213A
- Substrate — 0.2 mL
  Catalog Number MAK213B
- Neutrophil Elastase — 1 vial
  Catalog Number MAK213C
- Inhibitor Control, 3 mM SPCK — 100 µL
  Catalog Number MAK213D

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Fluorescence multiwell plate reader
- White flat-bottom 96-well plates. Cell culture or tissue culture treated plates are **not** recommended.
- Refrigerated microcentrifuge capable of RCF ≥ 14,000 × *g*

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C, protected from light.

Preparation Instructions

Briefly centrifuge the vials at low speed before opening. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Assay Buffer: Allow to come to room temperature prior to use.

Neutrophil Elastase Stock Solution: Reconstitute the Neutrophil Elastase vial with 220 µL of Assay Buffer. Mix well by pipetting. Aliquot and store at –70 °C. Use within 1 week of reconstitution.

Substrate and Inhibitor Control: Ready to use. Store at –20 °C.

Procedure

All samples should be run in duplicate.

Test Compounds
Dissolve candidate NE inhibitor compounds into an appropriate solvent. Dilute to 4× the final desired test concentration with Assay Buffer. Add 25 µL of diluted Test Compounds to separate wells of a white 96-well plate.

Inhibitor Control
Dilute Inhibitor Control Stock 1:25 with Assay Buffer. Add 25 µL of diluted Inhibitor Control to separate wells of the plate.

Enzyme Control
Add 25 µL of Assay Buffer to separate wells of the plate.

Note: The Enzyme Control must be set up each time the assay is run.

Background Control
Add 75 µL of Assay Buffer to separate wells of the plate.

Enzyme Solution Preparation

1. For each well (except Background Control wells), prepare 50 µL of Neutrophil Elastase Solution according to Table 1.

Table 1.

Reagent	Volume
Assay Buffer	48 µL
Neutrophil Elastase Stock Solution	2 µL

2. Add 50 µL of diluted Neutrophil Elastase Solution into each well labeled as Test Compound, Inhibitor Control, and Enzyme Control. Do **not** add to Background Control wells.
3. Mix well and incubate for 5 minutes at 37 °C. Protect the plate from light during the incubation. The volume of all wells including Test Compound(s), Inhibitor Control, Enzyme Control and Background Control at this step is 75 µL.

Reaction Mix

1. Mix enough reagent for the number of assays to be performed. For each well, prepare 25 µL of Reaction Mix according to Table 2.

Table 2.
Preparation of Reaction Mix

Reagent	Working Reagent
Assay Buffer	23 µL
Substrate	2 µL

2. Add 25 µL of the Reaction Mix into each reaction well including Test Compound(s), Inhibitor Control, Enzyme Control and Background Control.
3. Mix and measure the plate immediately.

Measurement
Measure the fluorescence (RFU) at λ_{Ex} = 400 nm /λ_{Em} = 505 nm in a microplate reader in kinetic mode for 30 minutes at 37 °C. Protect the plate from light during the incubation. It is recommended to take fluorescent readings every minute.

Results

1. For ALL wells, choose two time points (T_1 and T_2) in the linear range of the plot and obtain the corresponding RFU values (R_1 and R_2).
2. Calculate the changes in fluorescence (ΔRFU) generated by hydrolyzation of substrate:

   $$\Delta RFU = R_2 - R_1$$

3. Subtract the ΔRFU value of Background Control well from Test Compound(s), Inhibitor Control, and Enzyme Control wells.
4. Calculate the Relative Activity for each candidate inhibitor using the corrected ΔRFU values from Step 3 as follows:

   % Activity =

   $$\frac{\Delta RFU\ of\ Test\ Compound}{\Delta RFU\ of\ Enzyme\ Control} \times 100\%$$

   % Inhibition =

$$\left(1 - \frac{\Delta RFU\ of\ Test\ Compound}{\Delta RFU\ of\ Enzyme\ Control}\right) \times 100\%$$

Figure 1.
Neutrophil Elastase Activity Profile at Various Concentrations of SPCK.

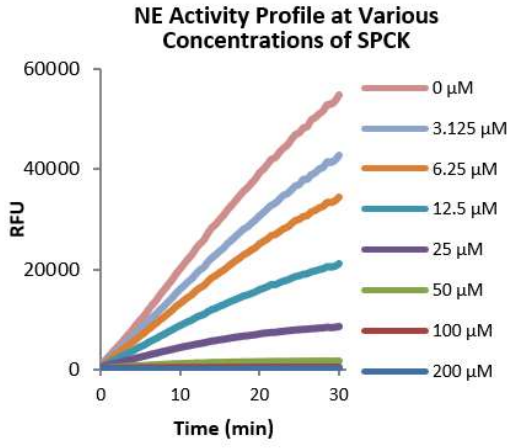
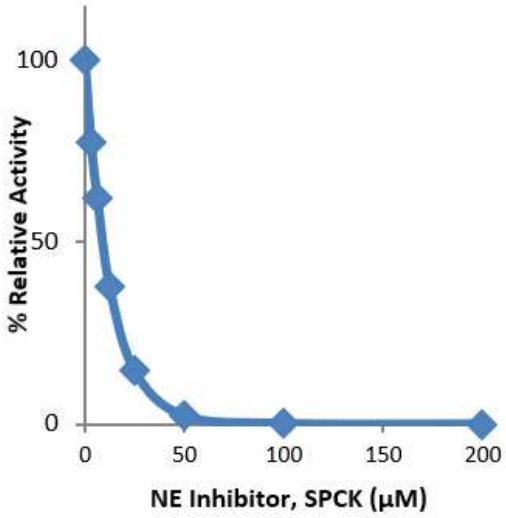


Figure 2.
Neutrophil Elastase Inhibition by SPCK. SPCK inhibits NE activity at different concentrations.



References

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3. Moroy, G. et al., Neutrophil elastase as a target in lung cancer. *Anticancer Agents Med. Chem.*, **12**, 565–579 (2012).

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