Neutrophil Elastase Inhibitor Screening Kit
Catalog Number MAK213
Storage Temperature –20 °C

Product Description
Neutrophil elastase (NE) is a serine protease that hydrolyzes proteins within the azurophilic granules of the 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 ($\lambda_{ex} = 400/\lambda_{em} = 505$ nm) proportional to the enzymatic activity present.

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

- **Assay Buffer**: 25 mL
  Catalog Number MAK213A

- **Substrate**: 0.2 mL
  Catalog Number MAK213B

- **Neutrophil Elastase**: 1 vL
  Catalog Number MAK213C

- **Inhibitor Control, 3 mM SPCK**: 100 µL
  Catalog Number MAK213D

Reagents and Equipment Required but Not Provided
- 96 well flat-bottom plate – It is recommended to use black plates with clear bottoms for fluorescence assays.
- Fluorescence multiwell plate reader

Precautions and Disclaimer
This product is 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.

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 before use.
- **Neutrophil Elastase (NE)** – Reconstitute 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.

Storage/Stability
The kit is shipped on wet ice and storage at –20 °C, protected from light, is recommended.

Procedure
Sample Preparation
Prepare a 4× Sample Inhibitor Solution by mixing sample inhibitors with Assay Buffer to 4× the final testing concentration. An initial concentrated inhibitor solution may be in a different solvent if the inhibitor is minimally soluble in the aqueous Assay Buffer.

For unknown inhibitor samples, it is suggested to test several inhibitor concentrations.

- An Inhibitor Control may be prepared by diluting 1 µL of Inhibitor Control with 24 µL of Assay Buffer.

To correct for background in samples, include a Sample Blank by omitting the Neutrophil Elastase. The Sample Blank readings can then be subtracted from the sample readings.

Prepare an Enzyme Control (uninhibited enzyme) by using Assay Buffer in place of sample inhibitor.
Add 25 μL of sample inhibitor (4× Sample Inhibitor Solution), Sample Blank (4× Sample Inhibitor Solution), Enzyme Control (Assay Buffer), or Inhibitor Control into duplicate wells of a 96 well plate.

**Assay Reaction**

1. Set up Inhibition Reaction Mixes according to the scheme in Table 1. 50 μL of the appropriate Inhibition Reaction Mix is required for each reaction (well).

**Table 1.**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Samples and Controls</th>
<th>Sample Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay Buffer</td>
<td>48 μL</td>
<td>50 μL</td>
</tr>
<tr>
<td>Neutrophil Elastase</td>
<td>2 μL</td>
<td>–</td>
</tr>
</tbody>
</table>

2. Add 50 μL of the appropriate Inhibition Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting. Incubate the plate at 37 °C for 5 minutes. Protect the plate from light during the incubation.

3. Set up an Enzymatic Reaction Mix according to the scheme in Table 2. 25 μL of the Enzymatic Reaction Mix is required for each reaction (well).

**Table 2.**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Samples, Controls, and Sample Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay Buffer</td>
<td>23 μL</td>
</tr>
<tr>
<td>Substrate</td>
<td>2 μL</td>
</tr>
</tbody>
</table>

4. Add 25 μL of the Enzymatic Reaction Mix to each reaction well. Mix well using a horizontal shaker or by pipetting.

5. Measure the fluorescence (FLU, $\lambda_{ex} = 400/\lambda_{em} = 505$ 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**

**Calculations**

Plot the fluorescence (FLU) for each well versus time.

Choose two time points (T1 and T2) in the linear range of the plot and obtain the slope for each well between T1 and T2. Determine the FLU at each time (FLU1 and FLU2) and use them to determine the slope of the plot (FLU/minute).

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

Subtract the slope of the Sample Blank from the slope of the samples to obtain the corrected measurement. Use the corrected measurement to determine the % Relative Inhibition.

**% Relative Inhibition**

$$\text{Slope} = (\text{FLU}_2 - \text{FLU}_1)/(T_2 - T_1) = \Delta \text{FLU/minute}$$

$$\% \text{ Relative Inhibition} = \left(\frac{\text{Slope}_{EC} - \text{Slope}_{SM}}{\text{Slope}_{EC}}\right) \times 100\%$$

where:

$\text{Slope}_{SM} =$ the slope of the Sample Inhibitor

$\text{Slope}_{EC} =$ the slope of the Enzyme Control

**Note:** Irreversible inhibitors that completely inhibit Neutrophil Elastase activity will have $\Delta \text{FLU} = 0$. The % Relative Inhibition will be 100%.

**Sample Calculation**

$\text{Slope}_{SM} = 0.435$ FLU/min

$\text{Slope}_{EC} = 0.755$ FLU/min

$$\% \text{ Relative Inhibition} = \left(\frac{0.755 - 0.435}{0.755}\right) \times 100\% = 42.4\%$$

**References**


# Troubleshooting Guide

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<th>Possible Cause</th>
<th>Suggested Solution</th>
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<td>Assay not working</td>
<td>Cold assay buffer</td>
<td>Assay Buffer must be at room temperature</td>
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<tr>
<td></td>
<td>Omission of step in procedure</td>
<td>Refer and follow Technical Bulletin precisely</td>
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<tr>
<td></td>
<td>Plate reader at incorrect wavelength</td>
<td>Check filter settings of instrument</td>
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<tr>
<td></td>
<td>Type of 96 well plate used</td>
<td>For Fluorometric assays, use black plates with clear bottoms</td>
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<tr>
<td>Samples with erratic readings</td>
<td>Samples prepared in different buffer</td>
<td>Use the Assay Buffer provided or refer to Technical Bulletin for instructions</td>
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<td>Repeat the sample homogenization, increasing the length and extent of homogenization step</td>
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<td>Samples used after multiple freeze-thaw cycles</td>
<td>Aliquot and freeze samples if needed to use multiple times</td>
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<td></td>
<td>Presence of interfering substance in the sample</td>
<td>If possible, dilute sample further</td>
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<tr>
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<td>Use of old or inappropriately stored samples</td>
<td>Use fresh samples and store correctly until use</td>
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<tr>
<td>Lower/higher readings in samples and standards</td>
<td>Improperly thawed components</td>
<td>Thaw all components completely and mix gently before use</td>
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<td>Use of expired kit or improperly stored reagents</td>
<td>Check the expiration date and store the components appropriately</td>
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<tr>
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<td>Allowing the reagents to sit for extended times on ice</td>
<td>Prepare fresh Reaction Mixes before each use</td>
</tr>
<tr>
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<td>Incorrect incubation times or temperatures</td>
<td>Refer to Technical Bulletin and verify correct incubation times and temperatures</td>
</tr>
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<td></td>
<td>Incorrect volumes used</td>
<td>Use calibrated pipettes and aliquot correctly</td>
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<tr>
<td>Non-linear standard curve</td>
<td>Use of partially thawed components</td>
<td>Thaw and resuspend all components before preparing the Reaction Mixes</td>
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<td></td>
<td>Pipetting errors in preparation of standards</td>
<td>Avoid pipetting small volumes</td>
</tr>
<tr>
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<td>Pipetting errors in the Reaction Mix</td>
<td>Prepare a Reaction Mixes whenever possible</td>
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<td>Air bubbles formed in well</td>
<td>Pipette gently against the wall of the plate well</td>
</tr>
<tr>
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<td>Standard stock is at incorrect concentration</td>
<td>Refer to the standard dilution instructions in the Technical Bulletin</td>
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<td>Unanticipated results</td>
<td>Samples measured at incorrect wavelength</td>
<td>Check the equipment and filter settings</td>
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<td>Samples contain interfering substances</td>
<td>If possible, dilute sample further</td>
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<tr>
<td></td>
<td>Sample readings above/below the linear range</td>
<td>Concentrate or dilute samples so readings are in the linear range</td>
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