ab118971

Neutrophil Elastase Inhibitor Screening Kit (Fluorometric)

Instructions for Use

For the rapid, sensitive and accurate screening of neutrophil elastase inhibitors.

This product is for research use only and is not intended for diagnostic use.

Version 7a, Last Updated 29 May 2025

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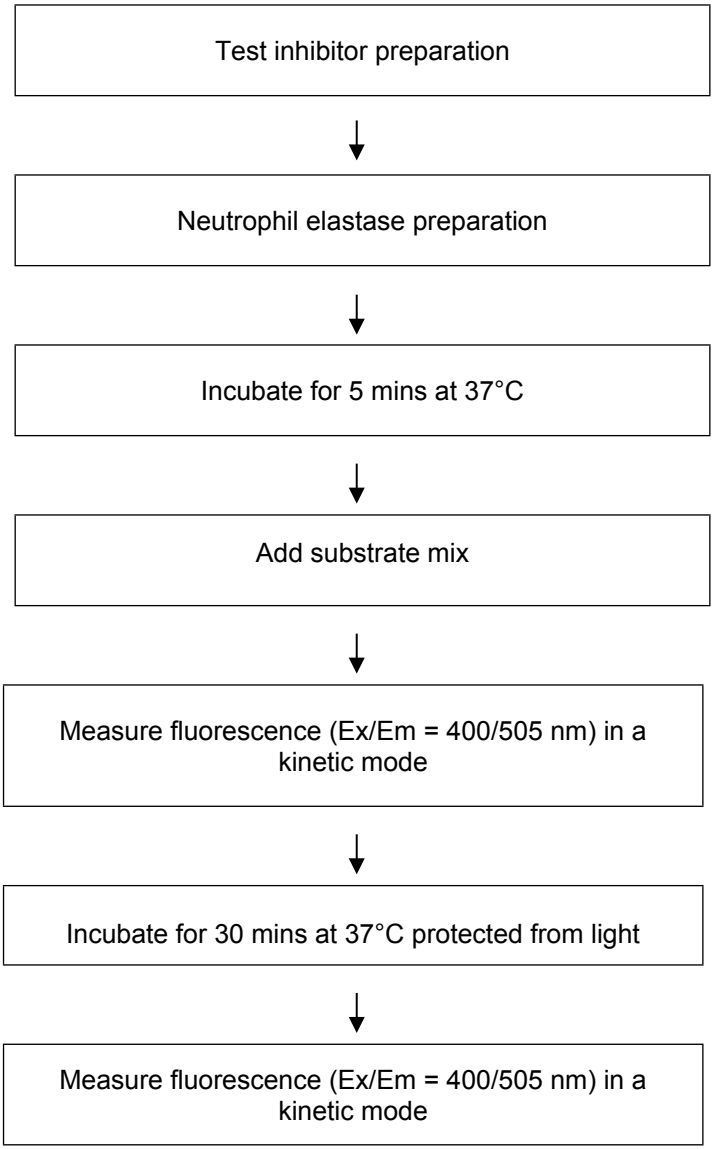
1. BACKGROUND

Neutrophil Elastase Inhibitor Screening Kit (ab118971) provides a rapid, simple, sensitive, and reliable test suitable as a high throughput screening assay of neutrophil elastase inhibitors. 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. For comparison of the relative efficacy of test inhibitors, a control inhibitor, SPCK (Ki = 10 µM for human leukocyte elastase) is included.

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.

2. ASSAY SUMMARY



3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at -20ºC in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 5.

Aliquot components in working volumes before storing at the recommended temperature. **Reconstituted components are stable for 2 months (except neutrophil elastase).**

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
Assay Buffer 40	25 mL	-20°C	-20°C
Substrate VI	200 µL	-20°C	-20°C
Elastase Enzyme	1 vial	-20°C	-80°C
Elastase Inhibitor	100 µL	-20°C	-20°C

PLEASE NOTE: Assay Buffer 40 was previously labelled as Assay Buffer XL and Assay Buffer. The composition has not changed.

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully perform this assay:

- Pipettes and pipette tips
- Fluorescent microplate reader – equipped with filter for Ex/Em = 400/505 nm.
- 96 well plate: white plate with flat bottom for fluorometric assay

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not use kit or components if it has exceeded the expiration date on the kit labels.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

8. TECHNICAL HINTS

- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Keep enzymes, heat labile components and samples on ice during the assay.
- Make sure all buffers and solutions are at room temperature before starting the experiment.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure complete removal of all solutions and buffers from tubes or plates during wash steps.
- Make sure you have the right type of plate for your detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on.

9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening.

9.1 **Assay Buffer 40:**

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.2 **Substrate VI:**

Ready to use as supplied. Equilibrate to room temperature before use. Aliquot substrate so that you have enough volume to perform the desired number of assays. Store at -20°C.

9.3 **Elastase Enzyme:**

Reconstitute the Elastase Enzyme Stock in 220 µL of Assay Buffer 40. Aliquot enzyme so that you have enough volume to perform the desired number of assays. Store at -80°C. Avoid repeated freeze/thaw cycles. Use within 1 week.

9.4 **Elastase Inhibitor:**

Ready to use as supplied. Aliquot inhibitor so that you have enough volume to perform the desired number of assays. Store at -20°C protected from light.

Prior to use, dilute sufficient Elastase Inhibitor 1:25 with Assay Buffer 40 e.g. 2 µL Elastase Inhibitor + 48 µL Assay Buffer 40.

10. TEST INHIBITOR PREPARATION

General Sample information:

- Samples should be dissolved in a suitable solvent.
- Dilute test inhibitor compounds to 4X desired test concentration in assay buffer.

11. ASSAY PROCEDURE and DETECTION

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all controls and samples in duplicate.**

11.1 NE Enzyme Preparation:

Using the Elastase Enzyme Stock solution, prepare 50 µL of diluted NE solution for each well (test compounds/inhibitors, inhibitor control and enzyme control wells):

Component	Volume (µL)
Assay Buffer 40	**48**
Elastase Enzyme/NE stock	**2**

11.2 Add 50 µl of diluted Elastase Enzyme solution into each well, labeled as Test Compound, Elastase Inhibitor, and Enzyme Control (Not Blank!)

11.3 Background Control: Add 75 µl Assay Buffer 40 into a Blank well as Background Control.

11.4 Set up Reaction wells:

Add test inhibitor, inhibitor control or assay buffer as follows:

	Test inhibitor ((µL)	Elastase Inhibtor (µL)	Assay Buffer 40 (µL)
Test inhibitor well	**25**	**0**	**0**
Inhibitor control well	**0**	**25**	**0**
Enzyme control well	**0**	**0**	**25**

Mix well and incubate at 37°C for 5 minutes.

∆**Note:** The volume of all wells including Test Compound(s), Elastase Inhibitor, Enzyme Control and Background Control is 75 µl.

11.5 Substrate Reaction Mix:

Prepare 25 µL of Substrate Reaction Mix for each reaction

Component	Fluorometric Reaction Mix (µL)
Assay Buffer 40	**23**
Substrate VI	**2**

Mix enough reagents for the number of assays (test compounds/inhibitors, standards (if using) and controls) to be performed. Prepare a master mix of the Substrate Reaction Mix to ensure consistency. We recommend the following calculation:

X µL component x (Number samples + controls +1)

11.6 Add 25 µL of Substrate Reaction Mix to all wells, including Test inhibitor, Elastase Inhibitor and Background control.

11.7 Immediately measure fluorescence at Ex/Em = 400/505 nm on a microplate reader in kinetic mode, for 30 minutes at 37°C (protected from light).

12. CALCULATIONS

- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

12.1 Average the duplicate reading for each test compound/inhibitor.

12.2 Draw the best smooth curve through these points to construct a curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your curve data (use the equation that provides the most accurate fit).

12.3 The RFU of fluorescence generated by hydrolyzation of substrate is $\Delta RFU = R_2 - R_1$. It is recommended to read kinetically and choose any two time points (t_1 and t_2) in the linear range of the plot to obtain the corresponding RFU values (R_1 and R_2).

12.4 Subtract the ΔRFU of Background Control well from Test Compound(s), Elastase Inhibitor, and Enzyme Control wells.

12.5 Calculate the relative activity for each test inhibitor as follows:

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

$$\% \text{ Inhibition} = \left(1 - \frac{(\Delta RFU \text{ of Candidate})}{(\Delta RFU \text{ of Enzyme Control})}\right) \times 100\ \%$$

13. TYPICAL DATA

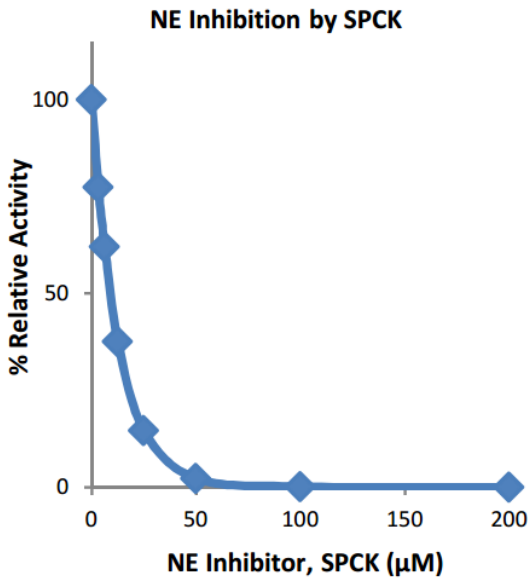


Figure 1. Typical neutrophil elastase inhibition calibration curve using fluorometric reading.

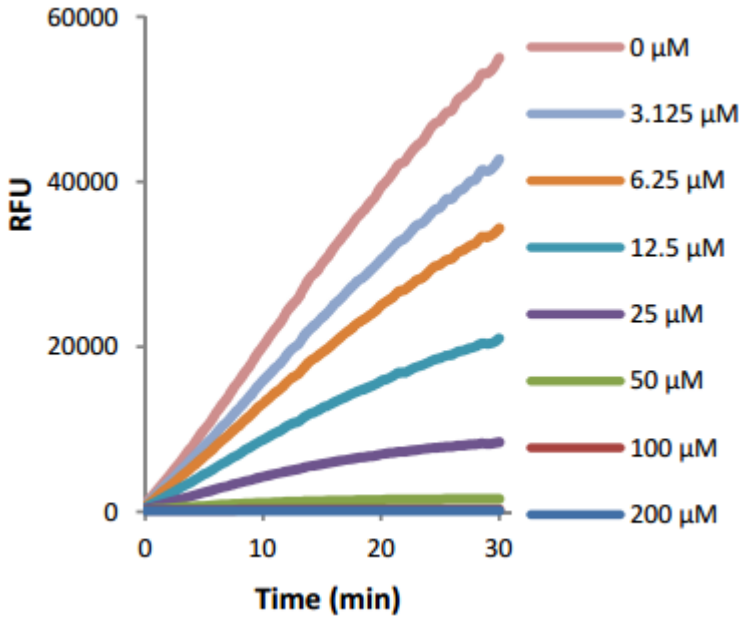


Figure 2: Neutrophil elastase activity profile with increasing concentrations of SPCK.

14. QUICK ASSAY PROCEDURE

***NOTE**: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.*

- Prepare NE enzyme, NE substrate, background contol and Elastase Inhibitor, (aliquot if necessary); get equipment ready.
- Add 50 µL diluted NE solution to all wells.
- Set up plate for test inhibitors (25 µL), Elastase Inhibitor (25 µL) and enzyme control (25 µL Assay Buffer 40), in duplicate.
- Mix and incubate plate at 37°C for 5 mins.
- Prepare 25 µL of Substrate Reaction Mix (Number samples + controls + 1).

Component	Fluorometric Reaction Mix (µL)
Assay Buffer 40	**23**
Substrate VI	**2**

- Add 25 µL Substrate Reaction Mix to each sample.
- Measure plate at Ex/Em= 400/505 nm (T_1).
- Incubate plate at 37°C 30 mins protected from light.
- Measure plate at Ex/Em= 400/505 nm (T_2).

15. TROUBLESHOOTING

Problem	Cause	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at room temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different 96-well plate	Colorimetric: Clear plates Fluorometric: black wells/clear bottom plate
Sample with erratic readings	Samples not deproteinized (if indicated on protocol)	Use PCA precipitation protocol for deproteinization
	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at -80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances; deproteinize samples
Lower/ Higher readings in samples and Standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol

Problem	Cause	Solution
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 µL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions on protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range

16. INTERFERENCES

These chemicals or biological materials will cause interferences in this assay causing compromised results or complete failure:

- RIPA: contains SDS which can destroy/decrease the activity of the enzyme.

17. NOTES

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)