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Product Information

Neutrophil Elastase Activity Assay Kit (Fluorometric)

Catalog Number **MAK246**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Neutrophil elastase (NE, EC 3.4.21.37, leukocyte elastase, ELANE, ELA2, elastase 2, neutrophil elaszym, or serine elastase) is a cytotoxic serine protease. Neutrophil elastase is stored in the azurophil granules of neutrophil granulocyte and is released following cell stimulation, e.g. by pathogens, immune complexes, or chemotactic agents (PMA).

When the extracellular NE concentration exceeds the buffering capacity of endogenous inhibitors, it causes degradation of a wide range of extracellular matrix proteins, including fibronectin, laminin, proteoglycans, collagens, and elastin. Extracellular elastase is implicated in the signs, symptoms, and disease progression of inflammatory lung disorders (such as cystic fibrosis, COPD, lung emphysema) via its role in the inflammatory processes, mucus overproduction, and lung tissue damage.

This Neutrophil Elastase Activity Assay Kit utilizes the ability of NE to proteolytically cleave a synthetic substrate and release a fluorophore, AFC, which can be easily quantified by fluorescence. This assay kit is simple, rapid, and can detect as low as 1 ng of neutrophil elastase in a variety of samples.

Components

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

NE Assay Buffer Catalog Number MAK246A	15 mL
NE Dilution Buffer Catalog Number MAK246B	1 mL
NE Substrate Catalog Number MAK246C	0.2 mL
NE Enzyme Standard (1 μg) Catalog Number MAK246D	1 vial

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – black plates are preferred for this assay.
- Fluorescence multiwell plate reader
- Red Blood Cell Lysis Buffer (BioVision Catalog Number 5830-100)

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

NE Assay Buffer – Bring to room temperature before use.

NE Enzyme Standard – Reconstitute with 10 μL of NE Dilution Buffer to prepare 100 ng/μL of stock solution. Mix well by pipetting up and down. Aliquot and store at –80 °C. Avoid repeated freeze/thaw cycles.

Storage/Stability

The kit is shipped on wet ice and storage at –20 °C, protected from light, is recommended. Allow assay buffer to warm to room temperature before use.

Procedure
All samples and standards should be run in duplicate.

Sample Preparations
Add 2–50 µL of purified enzyme, blood, or plasma sample per well of 96 well plate and adjust the volume to 50 µL/well with NE Assay Buffer.

Notes: To study chemotactic agents causing neutrophil stimulation and release of elastase, treating blood with Red Blood Cell Lysis Buffer (BioVision Catalog Number 5830-100) to isolate leukocytes prior to the treatment with a chemotactic agent is recommended.

(Optional) for samples having fluorescence background, prepare in parallel sample background control well(s) containing sample only and adjust the volume to 100 µL with NE Assay Buffer.

NE Standard Curve Preparation
Prepare NE Enzyme working solution (5 ng/µL) by adding 38 µL of NE Assay Buffer to 2 µL of the prepared NE Enzyme Standard (100 ng/µL). Add 0, 1, 2, 3, 4, and 5 µL of NE Enzyme working solution (5 ng/µL) into a series of wells in a 96 well plate to prepare 0, 5, 10, 15, 20 and 25 ng/well of NE Standard. Adjust the volume to 50 µL/well with NE Assay Buffer.

Note: Store any remaining NE Enzyme working solution at –80 °C and use within a week.

NE Substrate Mix
Prepare enough reagents for the number of assays to be performed. Prepare 50 µL of NE Substrate Mix for each Standard and sample well, see Table 1.

Table 1.
Preparation of NE Substrate Mix

Reagent	Volume
NE Assay Buffer	48 µL
NE Substrate	2 µL

Mix and add 50 µL of NE Substrate Mix into each Standard and sample well. Mix well.

Measurement
Measure fluorescence in kinetic mode for 10–20 minutes at 37 °C (λ_{ex} = 380 nm/λ_{em} = 500 nm). Choose two time points (T_1 and T_2) in the linear range of the plot and obtain the corresponding values for the fluorescence (RFU_1 and RFU_2).

Results
Calculations
Subtract 0 Standard reading from all readings. Plot the Neutrophil Elastase Standard Curve. Compare ΔRFU of the sample to the Neutrophil Elastase Standard Curve to obtain corresponding Neutrophil Elastase amount (B, in ng) and calculate the activity of Neutrophil Elastase in the sample as:

$$\text{NE Activity} = \frac{B}{V} \times \text{dilution factor} = (\text{ng/mL}) = (\mu\text{g/L})$$

B = NE amount from Standard Curve (ng)
V = The sample volume added into the reaction well (mL)

Note: If the sample background control reading is significant, subtract the sample background control reading from sample reading.

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay Not Working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	Black plates are preferred for this assay.
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly 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	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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