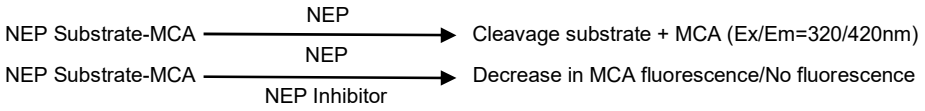
Neprilysin Inhibitor Screening Kit (Fluorometric) (#BN01153)

(Catalog # BN01153; 100 assays; Store at -20°C)

I. Introduction:

Neprilysin (NEP, EC 3.4.24.11), also known as neutral endopeptidase, enkephalinase, CD10, and common acute lymphoblastic leukemia antigen, is a type II zinc-containing transmembrane metalloproteinase. It is able to hydrolyze very important endogenous peptides, such as natriuretic atrial factor, enkephalins, substance P, bradykinin and amyloid beta-peptide. Thus, NEP is a potentially therapeutic target in important pathological conditions such as cardiovascular disease, prostate cancer, Alzheimer's disease and analgesia. NEP has also been used as a biological marker of a type of childhood leukemia called CALLA. NEP is currently a focus of interest in cardiovascular and neurobiological research. NEP inhibitors have shown promising results as potential treatment alternative for some conditions including hypertension, heart failure, renal failure, and improvement of β-cell function in obese type 2 diabetes mellitus. The combination of NEP inhibitor and angiotensin II type 1 receptor blockade has been used clinically to treat patients with chronic heart failure. Assay Genie's NEP Inhibitor Screening Kit can be used to screen for potential inhibitors of NEP activity. It utilizes the ability of an active NEP to cleave a synthetic MCA based peptide substrate to release a free fluorophore. The released MCA can be easily quantified using a fluorescence microplate reader. In the presence of an NEP specific inhibitor, the enzyme loses its activity which results in decrease of fluorescence intensity. The assay kit is simple and can be used to identify and characterize NEP inhibitors in a high-throughput format.



II. Applications:

- Screening/characterizing inhibitors/ligands of NEP

III. Kit Contents:

Components	BN01153	Cap Code	Part Number
NEP Assay Buffer	25 ml	WM	BN01153-1
NEP Enzyme (Lyophilized)	1 vial	Green	BN01153-2
NEP Substrate (in DMSO)	55 µl	Red	BN01153-3
NEP Inhibitor (10 mM Thiorphan)	10 µl	Brown	BN01153-4

IV. User Supplied Reagents and Equipment:

- 96-well white opaque plate
- Multi-well spectrophotometer (fluorescence plate reader)

V. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

- **NEP Assay Buffer:** Store at either 4ºC or -20ºC. Bring to room temperature before use.
- **NEP Enzyme:** Reconstitute NEP Enzyme in 110 µl NEP Assay Buffer and mix thoroughly. Aliquot and Store at -20°C. Avoid repeated freeze/thaw. Keep on ice while in use. Use within two months.
- **NEP Substrate and NEP Inhibitor:** Store at -20ºC. Bring to room temperature before use.

VI. Neprilysin Inhibitor Screening Protocol:

1. **Screening compounds, inhibitor control & blank control preparations:** Dissolve test sample at 100X in proper solvent. Further dilute to 10X the desired test concentration with NEP Assay Buffer. For inhibitor control: prepare a 100-fold dilution of Thiorphan (i.e. Add 1 µl of the Thiorphan stock solution to 99 µl NEP Assay Buffer and mix thoroughly. Add 10 µl diluted test sample or diluted Thiorphan or NEP Assay Buffer into wells assigned as test sample (Sample, S), Inhibitor Control (IC), or NEP Enzyme Control (EC) wells, respectively. Additional wells with serial dilutions of the test sample may be prepared at this time if desired, containing 10 µl per candidate well.

   Note: Various solvents, in which certain inhibitors are dissolved in, can affect the NEP enzyme activity. Prepare parallel well(s) as Solvent Control (SC) to test the effect of the solvent on enzyme activity.

2. **NEP Enzyme Working Solution Preparation:** Prepare a 10-fold dilution of NEP Enzyme (i.e. Dilute of 10 µl of NEP Enzyme with 90 µl of NEP Assay Buffer), mix thoroughly and keep on ice. Add 10 µl of Prepared NEP Enzyme Solution to each well containing test sample, Inhibitor Control NEP Enzyme Control and/or Solvent Control. Bring the volume of each well to 80 µl using NEP Assay Buffer, mix well and incubate at 37 °C for 10 min, avoid light.
3. **NEP Substrate Mix:** Prepare a 40-fold dilution of NEP Substrate Stock Solution (i.e. Dilute 5 µl of NEP Substrate with 195 µl of NEP Assay Buffer), vortex briefly and keep in ice. Add 20 µl of prepared NEP Substrate Solution to each well containing test sample, Inhibitor Control and NEP Enzyme Control.
4. **Measurement:** Measure fluorescence (Ex/Em= 320/420nm) in kinetic mode at 37 ºC for 60 min. Choose two points (t_1 and t_2) in the linear range of the plot and obtain the corresponding fluorescence values (RFU_1 and RFU_2).

5. **Calculation:** Calculate the slope for all samples, including Enzyme Control (EC), by dividing the net ΔRFU (RFU_2-RFU_1) values by the time Δt (t_2-t_1). use the value of SC well(s) instead of EC if it is significantly different from EC value). Calculate % Relative Inhibition as follows:

$$\% \text{ Relative Inhibition} = \frac{\text{Slope of [EC]} - \text{Slope of [S]}}{\text{Slope of EC}} \text{ X100}$$

$$\% \text{ Relative Activity} = \frac{\text{Slope of [S]}}{\text{Slope of EC}} \text{ X100}$$

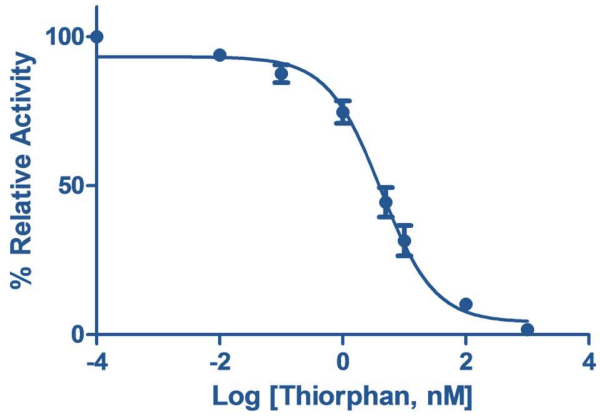


Figure: Inhibition of Neprilysin activity by Thiorphan. IC_{50} of Thiorphan was calculated to be 4.16 nM. Assay was carried out following the kit protocol.

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