ab284525 – Neprilysin Inhibitor Screening Kit (Fluorometric

For the screening of potential HDAC5 inhibitors.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab284525

Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Materials Supplied

Item	Quantity	Storage Condition
NEP Assay Buffer	25 mL	-20°C
NEP Enzyme	1 vial	-20°C
NEP Inhibitor	10 µl	-20°C
NEP Substrate	55 µl	-20°C

Materials Required, Not Supplied

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

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

Reagent Preparation

- Before using the kit, spin the tubes prior to opening.

NEP Assay Buffer: Store at either 4°C or -20°C. 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 -20°C. Bring to room temperature before use.

Assay Protocol

Test Compound preparation:

1. Dissolve the test compound at 100X in appropriate solvent.
2. Further dilute to 10X the desired test concentration with NEP Assay Buffer.
3. 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.
4. 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.
5. Additional wells with serial dilutions of the test sample may be prepared at this time if desired, containing 10 µl per candidate well.

***Δ 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.*

Reaction Mix:

1. Mix enough reagents for the number of assays to be performed, immediately before adding to the plate.
2. Prepare just enough for the number of reactions being rum immediately.
3. Keeps the vial containing the reaction mix on ice while adding it to the well of the 96 well plate. For each well, prepare 80 µl Mix containing:

	Reaction Mix
10X NEP Enzyme Solution	10 µl
NEP Assay Buffer	70 µl

4. Mix well and incubate for 10 min at 37°C, avoid light.

Substrate Mix:

1. 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) Mix enough reagents for the number of assays to be performed, vortex briefly and keep in ice.
2. Mix well and add 20 µl of the substrate mix to wells containing the enzyme control, inhibitor control, solvent control, and test compounds while the plate is still on ice.

Measurement

Start recording fluorescence at Ex/Em= 320/420 nm in kinetic mode at 37°C for 60 min.

Calculation:

1. Calculate the slope for all samples, including Enzyme Control (EC), by dividing the net ΔRFU (RFU2-RFU1) values by the time Δt (t2-t1). use the value of SC well(s) instead of EC if it is significantly different from EC value).
2. Calculate % Relative Inhibition as follows:

$$\% \text{ Inhibition} = \frac{slope\ of\ (EC) - slope\ of\ (S)}{slope\ of\ (EC)} \times 100$$

$$\% \text{ Relative activity} = \frac{slope\ of\ (S)}{slope\ of\ (S)} \times 100$$

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)

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