Version 1 Last updated 30 October 2018

ab241003
Neprilysin Assay Kit (Fluorometric)

For the detection of Neprilysin activity in purified enzyme preparations, tissues, adherent cells and suspension cells.

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

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1. Overview

Neprilysin Assay Kit (Fluorometric) (ab241003) provides a quick and easy method for monitoring NEP activity in a wide variety of samples.

This kit utilizes the ability of an active NEP to cleave a synthetic substrate (Abz-based peptide) to release a free fluorophore. The released Abz can be easily quantified using a fluorescence microplate reader. The substrate is specific to NEP and can differentiate the NEP activity from Trypsin and other structurally similar zinc metalloproteinase in biological samples such as Angiotensin-Converting Enzymes (ACE1, ACE2), Endothelin Converting Enzymes (ECE1, ECE2).

This kit is simple, specific and can detect as low as 20 µU/mg of NEP activity.

2. Protocol Summary

Prepare tissue or cell samples and positive/background control.

Prepare standard curve.

Prepare substrate stock solution and add to standards, positive control and sample wells.

Measure fluorescence (Ex/Em = 330/430 nm) immediately in kinetic mode for 1-2 hours at 37°C.

3. General guidelines, precautions, and troubleshooting

- Please observe safe laboratory practice and consult the safety datasheet.
- For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide: www.abcam.com/assaykitguidelines
- For typical data produced using the assay, please see the assay kit datasheet on our website.

4. Materials Supplied, and Storage and Stability

- Store kit at -20°C in the dark immediately upon receipt and check below in Section 6 for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.
- Aliquot components in working volumes before storing at the recommended temperature.

Item	Quantity	Storage condition
NEP Assay Buffer	40 mL	-20°C
Neprilysin (Lyophilized)	1 vial	-20°C
NEP Substrate (in DMSO)	15 µL	-20°C
Abz-Standard (1 mM)	100 µL	-20°C

5. Materials Required, Not Supplied

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

- 96-well white opaque plate
- Multi-well spectrophotometer

6. Reagent Preparation

- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Prepare only as much reagent as is needed on the day of the experiment.

6.1 NEP Assay Buffer:

Ready to use as supplied. Bring to room temperature before use. Store at 4°C or -20°C.

6.2 Neprilysin (Lyophilized):

Reconstitute Neprilysin in 500 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.

6.3 NEP Substrate (in DMSO):

Ready to use as supplied. Store at -20°C, protect from light.

6.4 Abz-Standard (1 mM):

Ready to use as supplied. Store at -20°C, protect from light.

7. Standard Preparation

– Always prepare a fresh set of standards for every use.

7.1 Prepare a 100 µM solution of Abz-Standard by diluting 10 µL of 1 mM Abz-Standard with 90 µL of NEP Assay Buffer.

7.2 Add 0, 2, 4, 6, 8, and 10 µL of 100 µM Abz-Standard into a series of wells in a 96-well white plate and adjust the final volume to 100 µL/well with NEP Assay Buffer to generate 0, 200, 400, 600, 800 and 1000 pmol/well of Abz-Standard respectively, mix well.

Standard #	Abz 100 µM Standard (µL)	NEP Assay Buffer (µL)	NADH Standard pmol/well
1	0	100	0
2	2	98	200
3	4	96	400
4	6	94	600
5	8	92	800
6	10	90	1000

8. Sample Preparation

8.1 Homogenize tissue (~100 mg) or pelleted cells (~1-2 X10^6) with 400 µL of iced-cold NEP Assay Buffer containing protease inhibitors (we suggest use 1 mM PMSF and 10 µg/ml Aprotinin) and keep on ice for 10 min.

8.2 Centrifuge samples at 12,000 x *g* at 4°C for 10 min. and collect the supernatant.

8.3 Add 1-10 µL of sample into desired well(s) in a 96-well white plate labeled as Sample and Sample Background Control.

8.4 For positive control, add 4-10 µL of reconstituted Neprilysin into desired well(s).

8.5 Adjust the volume of Positive Control, Sample Background Control and Sample wells to 90 µL/well with NEP Assay Buffer.

Δ Note:

- Neprilysin is zinc-containing transmembrane metalloproteinase. Tested samples should not contain EDTA/EGTA.
- Tissue or cell lysates of more than 15 µg of total protein/well might suppress the enzymatic activity of NEP with the provided substrate. For samples having high protein concentration, dilute the sample with NEP Assay Buffer and use 3-5 different amounts of the diluted samples per well to ensure the change of velocity of the readings is within the linear range.
- Some protease inhibitors might suppress the enzymatic activity of NEP with the provided substrate. We suggest use freshly prepared PMSF and Aprotinin.
- Equilibrate the NEP Assay Buffer to 37°C before adding to the wells.

9. Assay Procedure

NEP Substrate Solution Preparation:

9.1 Prepare a 100-fold dilution of NEP Substrate Stock Solution (i.e. Dilute 2 µL of NEP Substrate with 198 µL of NEP Assay Buffer), vortex briefly.

9.2 Add 10 µL of Diluted NEP Substrate Solution to each well containing Test Sample(s) and NEP positive control(s).

9.3 For Sample Background Control, add 10 µL of NEP Assay Buffer.

9.4 The total volume for each well is 100 µL.

Δ Note: Equilibrate the Substrate Solutions to 37°C before adding to the wells.

9.5 Measure fluorescence (Ex/Em= 330/430nm) in kinetic mode at 37°C for 1-2 hours.
Δ Note: Incubation time depends on the NEP activity in the samples. Longer incubation time may be required for samples having low NEP activity.

9.6 We recommend measuring the reaction progress in kinetic mode and choosing two time points (T1 and T2) in the linear range to calculate the NEP activity of the samples. The Abz Standard Curve can be read in endpoint mode (i.e. at the end of incubation time).

10. Data Analysis

10.1 Subtract 0 Standard Reading from all Standard Readings.

10.2 Plot the Abz Standard Curve and obtain the slope of the curve (ΔRFU/pmol); apply Sample ΔRFU and Sample Background Control ΔRFU to Abz Standard Curve to obtain the corresponding amount of Abz formed.

10.3 Calculate the background-corrected sample ΔRFU (B, in pmol) by subtracting the amount of Abz formed by Sample Background Control from the amount of Abz formed by Sample and calculate the activity of NEP activity in the sample as:

Sample NEP Activity = **B/ΔT x V x D** = pmol/min/mL = µU/mL

Where:

B is Abz amount in the sample well from Standard Curve (pmol).
ΔT is reaction time (min).
V is sample volume added into the reaction well (mL)
D is sample dilution factor (D=1 when samples are undiluted)

NEP Activity in samples can also be expressed in U/mg of protein.

Unit Definition: One unit of NEP activity is the amount of enzyme that catalyzes the release of 1 µmol of Abz per min from the substrate under the assay conditions at 37°C.

11. Typical Data

Typical data provided **for demonstration purposes only**.

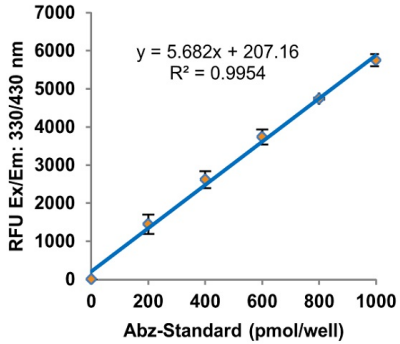

Figure 1. Abz Standard Curve.

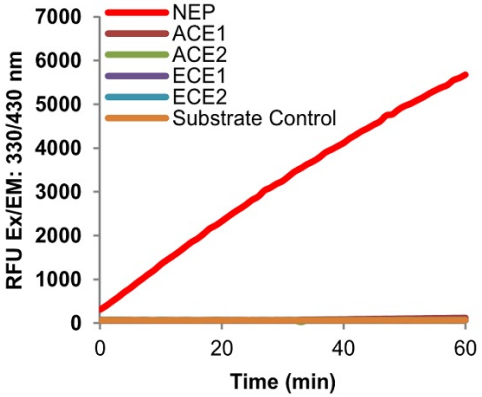

Figure 2. Measurement of purified NEP (10 ng), ACE1 (150 ng), ACE2 (0.5 ng), ECE1 (15 ng) and ECE2 (20 ng) activities using our proprietary substrate. The kit can efficiently distinguish NEP activity from other zinc metalloproteases in biological samples.

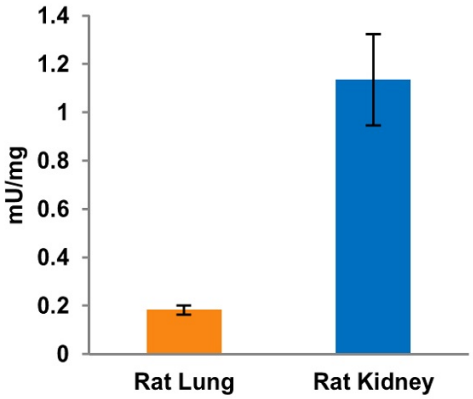

Figure 3. Measurement of NEP activity in rat lung (10 µg protein) and rat kidney (8.5 µg protein).

13. Notes

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