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ab273322 Ethanolamine Kinase (ETNK) Activity Assay Kit (Fluorometric)

View Kit datasheet: https://www.abcam.com/ab273322
(use https://www.abcam.cn/ab273322 for china, or
https://www.abcam.co.jp/ab273322 for Japan)

For the determination of Ethanolamine Kinase activity.

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

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1. Overview

Ethanolamine Kinase (ETNK) Activity Assay Kit (Fluorometric) (ab273322) is suitable for detecting ETNK enzyme activity in different tissues and it can detect as low as 0.1 mU of ETNK in biological samples. Samples include cell culture, crude lysate and animal tissues (heart, liver, kidney, lung, and muscle, etc.).

In the assay ENTK phosphorylates Ethanolamine producing Phosphoethanolamine and ADP. The produced ADP is then detected with a set of enzymatic reactions that generate fluorescent product (Ex/Em: 535/587 nm). The fluorescence signal is directly proportional to the generated ADP.

2. Protocol Summary

Prepare all reagents, samples and controls.

Prepare standards.

Add all samples to the appropriate wells.

Add Reaction Mix and Background Mix to the appropriate wells.

Measure fluorescence in kinetic mode for 60 mins at 37°C.

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.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C in the dark immediately upon receipt.

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

Aliquot components in working volumes before storing at the recommended temperature.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- 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.

6. Materials Supplied

Item	Quantity	Storage temperature (before prep)
ENTK Assay Buffer	25 mL	-20°C
ENTK Substrate	1 vial	-20°C
ENTK Converter (Lyophilized)	1 vial	-20°C
ENTK Developer (Lyophilized)	1 vial	-20°C
ENTK Probe	0.2 mL	-20°C
ADP Standard (1 µmol)	1 vial	-20°C
ETNK Enzyme	20 µL	-20°C

7. Materials Required, Not Supplied

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

- 96-well black flat-bottom plate
- Multi-well spectrophotometer.

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.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- 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 all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature

  .

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening. The kit components are stable for one year when stored as recommended.

9.1 **ENTK Assay Buffer:**
Ready to use as supplied. Warm bottle to room temperature before use. Store at 4°C.

9.2 **ENTK Substrate:**
Add 220 µl of water to the vial. Warm vial to room temperature before use. Store at -20°C. Use within 2 months.

9.3 **ENTK Converter (Lyophilized):**
Reconstitute ETNK Converter with 220 µl of Assay Buffer separately. Warm component to room temperature before use. Keep on ice after use. Store at -20°C. Use within 2 months.

9.4 **ENTK Developer (Lyophilized):**
Reconstitute ETNK Developer with 220 µl of Assay Buffer separately. Warm component to room temperature before use. Keep on ice after use. Store at -20°C. Use within 2 months.

9.5 **ENTK Probe:**
Ready to use as supplied. Warm probe at room temperature. Mix well. Store at –20°C and protect from light. Use within 2 months.

9.6 **ADP Standard:**
Dissolve in 2 ml of water to generate 500 µM ADP stock. Keep on ice while in use. Store at -20°C. Use within 2 months.

9.7 **ETNK Enzyme:**
Ready to use as supplied. Keep on ice while in use. Aliquot and store at -20°C. Avoid repeated freeze thaw.

10. Sample Preparation

Tissue/cell lysate preparation:

10.1 Homogenize tissue (10 mg) or cells (1 x 10^6) with 100 µl of cold Assay Buffer. Keep on ice for 15 mins.

10.2 Centrifuge at 10,000 *x g* for 15 mins and transfer the sample supernatant to a new tube.

10.3 For sample and sample background: prepare duplicates by adding 5-20 µl of the supernatant to the designated wells in a 96-well black flat-bottom plate.

10.4 Adjust the volume to 50 µl using ETNK Assay Buffer.

10.5 For Enzyme control: add 4 µl of ETNK enzyme into a desired well in the plate.

10.6 Adjust the volume to 50 µl using ETNK Assay Buffer.

11. Standard Curve

11.1 Dilute 500 µM ADP 10-fold (e.g. 100 µl in 900 µl of ddH_2O) to prepare 50 µM ADP.

11.2 Add 0, 5, 10, 15 and 20 µl of 50 µM ADP into the desired wells to generate 0, 0.25, 0.5, 0.75 and 1 nmol of ADP standards respectively.

11.3 Adjust the volume to 50 µl using ETNK Assay Buffer.

Standard #	50 µM ADP - Standard (µL)	ADP Assay Buffer (µL)	ADP (nmol/well)
1	20	30	1.00
2	15	35	0.75
3	10	40	0.50
4	5	45	0.25
5	0	50	0

12. Assay Procedure

– Keep on ice while in use

Reaction mix:

12.1 Prepare enough reagents for the number of assays to be performed.

12.2 Prepare 50 µl of Reaction Mix and 50 µl of Sample Background Control Mix as indicated in the table below:

	Reaction Mix (µl)	Background Mix (µl)
ETNK Assay Buffer	43.5	45.5
ETNK Substrate	2	---
ETNK Converter	2	2
ETNK Developer	2	2
Probe	0.5	0.5

12.3 Add 50 µl of Reaction Mix to every well containing Standards, Sample tests, and Positive Control.

12.4 Add 50 µl of Sample Background Control to the wells designated as sample background controls.

12.5 Measure fluorescence (Ex/Em = 535/587 nm) in kinetic mode for 60 mins at 37°C.

13. Calculations

13.1 Plot the ADP standard curve. If the sample background is significant, subtract the background control reading from its paired sample reading.

13.2 Calculate the ETNK activity of the test sample:

ΔRFU = RFU2 – RFU1 for a time interval (t2 – t1) such that both time points fall in the linear portion of the reaction.

13.3 Apply the ΔRFU to the ADP standard curve to get A nmol of ADP generated during the reaction time (Δt = t2 – t1).

13.4 To calculate the specific ACE2 activity of Sample, subtract ΔRFU of Negative Control (ΔRFU_{NC}) from Sample (ΔRFU_S).

$$Sample\ ETNK\ specific\ activity\ = \frac{B\ xD}{(\Delta t\ x\ P)}\ = (mU/mg)$$

B = ADP from standard curve (nmol)
Δt = Reaction time (min)
D = Dilution factor
P = Protein used (mg)

Unit definition:

One unit of ETNK is 1 µmole of ADP generated per min at pH 7 and 37°C.

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

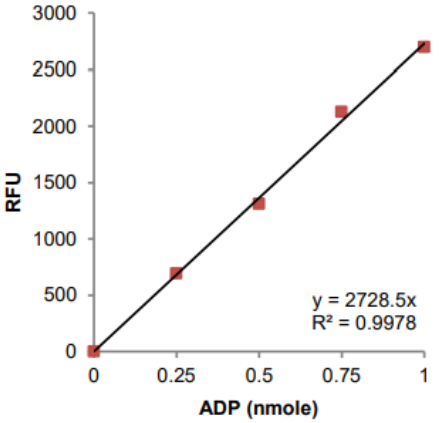

Figure 1. ADP Standard Curve.

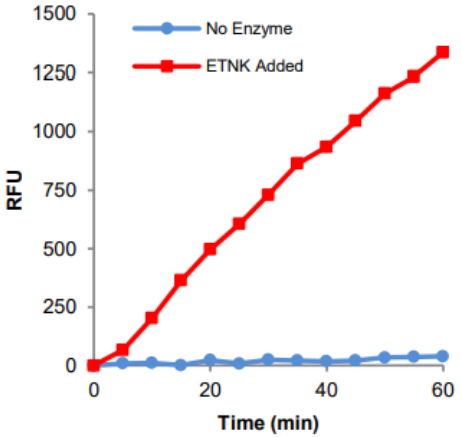

Figure 2. Reaction curves of ETNK vs. no enzyme control in the assay.

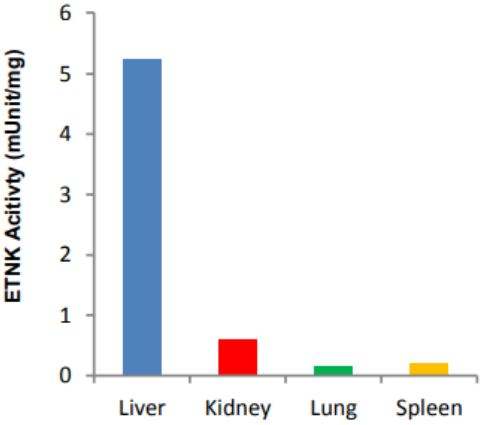

Figure 3. Specific ETNK2 activity in different mouse tissues determined using the kit protocol.

15. FAQ / Troubleshooting

General troubleshooting points are found at www.abcam.com/assaykitguidelines.

16. Notes

Technical Support

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