

Ethanolamine Kinase (ETNK) Activity Fluorometric Assay

(Catalog: TBS2209, 100 Assays, Store at -20°C)

DESCRIPTION

Ethanolamine kinase (ETNK, EC 2.7.1.82) catalyzes the phosphorylation of ethanolamine to phosphoethanolamine (P-Etn), a key step in phospholipid biosynthesis. Reduced ETNK1 activity leads to lower P-Etn, which removes its inhibition of mitochondrial complex II. This causes mitochondrial hyperactivation, increases reactive oxygen species (ROS), DNA damage, and reduces cell viability. ETNK could be a candidate for further study in Alzheimer's disease and other diseases with kinase-related networks and phospholipid signaling pathways.

Tribioscience's ETNK Activity Assay is designed to be a flexible, accurate, simple, and time-saving method in which ETNK catalyzes ethanolamine into P-Etn and ADP, resulting in an intermediate formation through a coupled enzyme reaction. It is catalyzed and reacts with the probe, to generate a fluorometric (Ex/Em=530/590 nm) intensity proportional to the ETNK activity which can be measured.

SYNONYMS

EK, ETNK1, EK11, ethanolamine phosphokinase

APPLICATIONS

ETNK activity in cells and various biological samples.

KIT CONTENTS

Name	Size (100 tests)
Assay Buffer	20 mL
ETNK Enzyme Mix	14 µL
ETNK Substrate	50 µL
Assay Co-substrate	14 µL
Assay Cofactor	40 µL
Assay Developer	35 µL
ETNK Probe	240 µL
ETNK Positive Control	160 µL
ADP Standard (5 mM)	50 µL

STORAGE AND HANDLING

Store the kit at -20°C. The shelf life is 12 months.

ASSAY PROTOCOL

Except Enzyme, warm all the components to room temperature before use. Briefly centrifuge all small vials prior to opening.

1. Sample Preparation:

Tissue (1-10 mg) or cells (1×10^6) can be lysed in 100 µL of Assay Buffer. Centrifuge ice cold at 15,000 x g for 2 minutes to pellet insoluble materials and collect supernatant. For more accurate assay, the sample should be quickly frozen using liquid N₂ or dry ice if it is to be assayed later.

2. Standard Curve Preparations:

2.1 Label 1.5mL tubes 1-8 for a standard curve preparation as displayed in Table 1.

Add 384µL of 1x Assay Buffer to Std 1 and 200µL to Std 2-8.

2.2 Add 16 µL of 5 mM ADP Stock solution to Std1 and fully mix.

2.3 Carry out a 2x serial dilution sequentially for Std 2-7 by transferring 200µL of Std1 to Std2 and afterwards. Leave Std 8 as the 0 standard (the assay buffer alone as blank). The standards concentrations are 200, 100, 50, 25, 12.5, 6.25, 3.13, and 0 µM for Std 1-8.

Table1: Assay Standard Preparation

Tubes	ADP (µL)	Buffer	ADP (µM)
1	16 µL 5 mM stock	384 µL	200
2	200 µL of Tube#1	200 µL	100
3	200 µL of Tube#2	200 µL	50
4	200 µL of Tube#3	200 µL	25
5	200 µL of Tube#4	200 µL	12.5
6	200 µL of Tube#5	200 µL	6.25
7	200 µL of Tube#6	200 µL	3.13
8	0	200 µL	0

3. Load Samples: Add 50 µL/well standards or samples or ETNK positive control to 96-well plate (Note: use black plate for fluorometric assay).

4. ETNK Reaction Mix1:

Prepare mix1 for 100 wells test by adding 35 µL of ETNK substrate to 1965 µL of assay buffer. Adding to 20 µL mix1 to each well except standards. Add 20 µL assay buffer to each standard instead of mix1.

5. Incubate the plate at 37°C for 1 hour as T1.

6. ETNK Reaction Mix2:

Prepare 3000 µL of reaction mix2 for 100 wells test as Table 2:

Table 2: Reaction Mix Preparation

Reaction Components	Volume
Assay Buffer	2722 µL
Co-substrate	10 µL
Assay Cofactor	29.5 µL
ETNK Enzyme mix	11.5 µL
Assay developer	27 µL
ETNK probe	200 µL
Final Volume (µL)	3000 µL

Add 30 µL of the ETNK Reaction Mix2 to each well containing the ADP Standards, ETNK Positive Control, and test samples. Tap plate lightly to mix. Protect the plate from light during incubation.

7. Measurement:

Measure the absorbance value at fluorescence at Ex/Em = 530/590 nm in kinetic model for 5-20 min after adding mix2 as T2 at 37°C (Note: the incubation time is dependent on the ETNK activity in the samples).

8. Calculation:

Correct background by subtracting the value of the 0 standard (blank) from all readings to get ΔRFU. Plot the standard curve. Apply the corrected sample value to the standard curve to get B µM of ADP generated by ETNK during the reaction time. (e.g. The formular on standard curve is $Y=mB+c$, Y as sample

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ΔRFU , then $B = (Y - c) / m$, c : the Y-intercept, a : the slope of the line).

Sample ETNK Activity = $N * B / (T * V) = \mu M / \mu l$ or = $U / \mu l$.

Where: B = ADP amount from Standard Curve (μM)

V = Sample volume in the reaction well (μL)

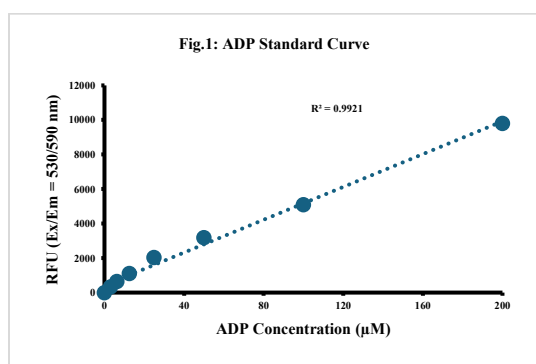
N = Dilution Factor

T = incubation time ($T_1 + T_2$ total minutes)

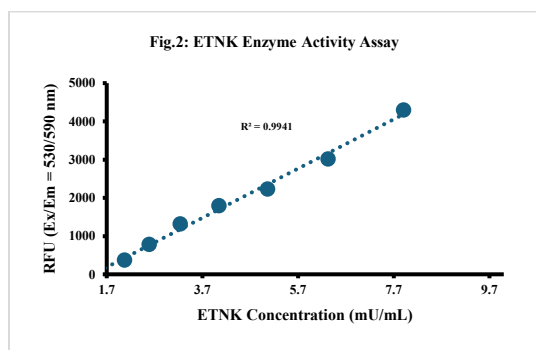
Unit Definition: One unit (U) is the amount of enzyme that will generate 1.0 μmol of ADP per minute at pH7.5 at 37 °C.

9. Typical Standard Curve for Fluorometric Assays:

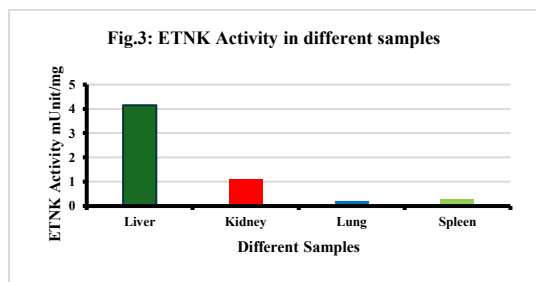
The typical standard curves are shown in Fig. 1. The Typical standard curves are only used as a reference for assay. It cannot be used for real standard curve for assay.



The enzyme activity assay curves are shown in Fig. 2



The different tissues of enzyme activity assay curves are shown in Fig. 3



RELATED PRODUCTS

ETNK1 Protein, Bioactive (TBP0249)
 CDK4 Fluorometric Assay (TBS2127)
 CDK6 Fluorometric Assay (TBS2128)
 EGFR Activity Assay (TBS2048)
 LDH Cytotoxicity Assay (TBS2002)
 ATP Colorimetric/Fluorometric Assay (TBS2010)
 ADP Colorimetric/Fluorometric Assay (TBS2020)
 XTT Cell Viability Assay (TBS2021)
 Cell Count Kit -8 (TBS2022)
 Thiol Fluorometric Assay (TBS2026)
 GSH Assay (TBS2028)
 NAD/NADH Colorimetric Assay (TBS2029)
 Caspase-3 Colorimetric Assay (TBS2030)
 Pyruvate Colorimetric Assay (TBS2023C)
 Glycerol Colorimetric Assay (TBS2204C)
 Triglyceride Colorimetric Assay (TBS2205C)
 NNMT Inhibitor Screening Assay (TBS2097)
 NNMT Activity Fluorometric Assay (TBS2098)
 G6PDH Activity Colorimetric Assay (TBS2102)
 Cytochrome c Reductase Activity Assay (TBS2116)
 Mitochondria Complex 1 Activity Assay (TBS2017)
 Mitochondria Oxidase Activity (TBS2105)
 Mitochondrial Membrane Potential Assay (TBS2049)
 Cytochrome C Oxidase (Complex IV) Activity Assay (TBS2115)

This product is for research only.