

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

1/19

(Catalog # K498-100; 100 assays, Store kit at -20°C)

### I. Introduction:

Ethanolamine kinase (ETNK; E.C. 2.7.1.82), catalyzes the ATP-dependent phosphorylation of free intracellular ethanolamine and is the first committed step of the *de novo* CDP-ethanolamine pathway for phosphatidylethanolamine (PE) biosynthesis in mammalian tissues. PE is a major phospholipid component of cell membranes and it is involved in diacylglycerol and G protein-dependent signal transduction. ETNK plays a critical role in cell growth and it may be associated with abnormal choline and ethanolamine phospholipid metabolism in breast, prostate, ovarian and cervical cancers. Two isoforms of ethanolamine kinase, ETNK1 and ETNK2, have been identified in mammalian cells and sharing > 50% homology. In BioVision's Ethanolamine Kinase Activity Assay Kit, ETNK 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. BioVision's ETNK assay 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.



### II. Application:

- Measurement of ETNK activity in various tissues
- Analysis of phosphatidylethanolamine and phospholipid biosynthesis

### III. Sample Type:

- Cell culture, crude lysate and animal tissues (heart, liver, kidney, lung, and muscle, etc.).

### IV. Kit Contents:

Components	K498-100	Cap Code	Part Number
ENTK Assay Buffer	25 ml	WM	K498-100-1
ENTK Substrate	1 vial	Brown	K498-100-2
ENTK Converter	1 vial	Purple	K498-100-3
ENTK Developer	1 vial	Green	K498-100-4
ENTK Probe	0.2 ml	Red	K498-100-5
ADP Standard (1 μmol)	1 vial	Yellow	K498-100-6
ETNK Enzyme	20 μl	Blue	K498-100-7

### V. User Supplied Reagents and Equipment:

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

### VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C. The kit components are stable for one year when stored as recommended. Briefly centrifuge small vials at low speed prior to opening. Read the entire protocol before performing the experiment.

- **ENTK Assay Buffer:** Ready to use as supplied. Warm bottle to room temperature before use. Store at 4°C.
- **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.
- **ENTK Converter and Developer:** Reconstitute ETNK Converter and Developer with 220 μl of Assay Buffer separately. Warm each component to room temperature before use. Keep on ice after use. Store at -20°C. Use within 2 months.
- **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.
- **ADP Standard:** Dissolve in 2 ml of water to generate 500 μM ADP stock. Keep cold while in use. Store at -20°C. Use within 2 months.
- **ETNK Enzyme:** Ready to use as supplied. Keep cold while in use. Aliquot and store at -20°C. Avoid repeated freeze thaw.

### VII. ETNK Fluorometric Assay Protocol:

- Sample Preparation:** Homogenize tissue (10 mg) or cells ( $1 \times 10^6$ ) with 100 μl of cold Assay Buffer. After homogenization, keep on ice for 15 min. Centrifuge at 10,000 x g for 15 min and transfer the sample supernatant to a new tube. 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. Adjust the volume to 50 μl using ETNK Assay Buffer. **For Enzyme control:** add 4 μl of ETNK enzyme into a desired well in the plate. Adjust the volume to 50 μl using ETNK Assay Buffer.
- ADP Standard:** Dilute 500 μM ADP 10-fold (eg. 100 μl in 900 μl of ddH<sub>2</sub>O) to prepare 50 μM ADP. 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 nmole of ADP standards respectively. Adjust the volume to 50 μl using ETNK Assay Buffer.
- Reaction Mix:** Mix enough reagents for the number of assays to be performed. Prepare 50 μl of Reaction Mix and 50 μl of Sample Background Control Mix as indicated in the table below:

	<u>Reaction Mix</u>	<u>Sample Background</u>
ETNK Assay Buffer	43.5 $\mu$ l	45.5 $\mu$ l
ETNK Substrate	2 $\mu$ l	--
ETNK Converter	2 $\mu$ l	2 $\mu$ l
ETNK Developer	2 $\mu$ l	2 $\mu$ l
Probe	0.5 $\mu$ l	0.5 $\mu$ l

Add 50  $\mu$ l of Reaction to every well containing Standards, Sample tests, and Positive Control and 50  $\mu$ l of Sample background Control to the wells designated as sample background controls.

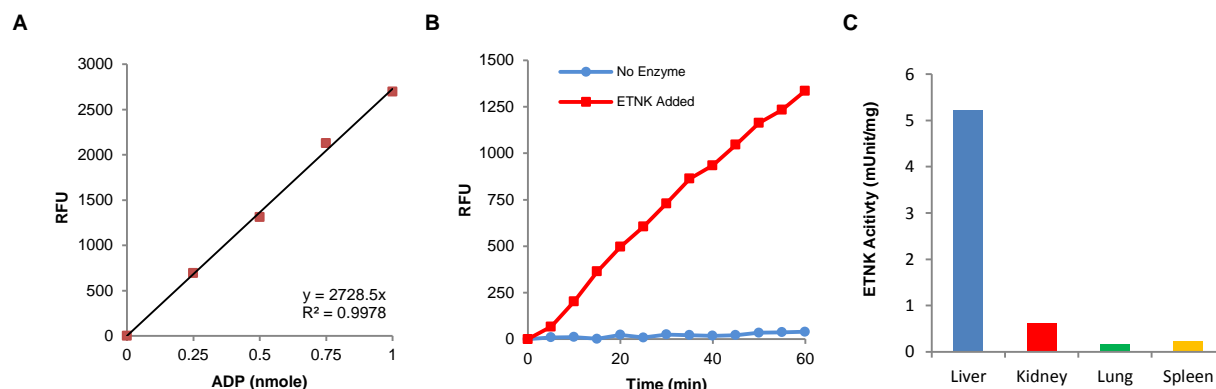
**4. Measurement:** Measure fluorescence at Ex/Em 535/587 nm in kinetic mode at 37°C for 60 min.

**5. Calculation:** Plot the ADP standard curve. If the sample background is significant, subtract the background control reading from its paired sample reading. Calculate the ETNK activity of the test sample:  $\Delta\text{RFU} = \text{RFU}_2 - \text{RFU}_1$  for a time interval ( $t_2 - t_1$ ) such that both time points fall in the linear portion of the reaction. Apply the  $\Delta\text{RFU}$  to the ADP standard curve to get A nmol of ADP generated during the reaction time ( $\Delta t = t_2 - t_1$ ).

$$\text{Specific ETNK Specific Activity} = B \times D / (\Delta t \times P) \text{ (mU/mg)}$$

Where: B = ADP from standard curve (nmol)  
 $\Delta t$  = Reaction time (min)  
 D = Dilution factor  
 P = Protein used (mg)

**Unit Definition:** One unit of ETNK is 1  $\mu$ mole of ADP generated per min at pH 7 and 37°C.



**Figure A.** ADP Standard Curve. **Figure B.** Reaction curves of ETNK vs. no enzyme control in the assay. **Figure C.** Specific ETNK2 activity in different mouse tissues determined using the kit protocol.

#### VIII. RELATED PRODUCTS:

Ethanolamine Kinase 2, human recombinant (P1158)  
 Phospholipid Assay Kit (Colorimetric/Fluorometric) (K351)  
 Phospholipase D (PLD) Activity Colorimetric Assay Kit (K725)  
 Pyruvate Colorimetric/Fluorometric Assay Kit (K609)  
 Citrate Colorimetric/Fluorometric Assay Kit (K655)  
 Citrate Synthase Activity Colorimetric Assay Kit (K318)  
 Succinate (Succinic Acid) Colorimetric Assay Kit (K649)

Choline Kinase B, Human Recombinant (P1220)  
 Phospholipase A2 Activity Assay Kit (Fluorometric) (K400)  
 Malate Colorimetric Assay Kit (K637)  
 PicoProbe™ Acetyl-CoA Fluorometric Assay Kit (K317)  
 Oxaloacetate Colorimetric/Fluorometric Assay kit (K659)  
 Isocitrate Colorimetric Assay Kit (K656)

**FOR RESEARCH USE ONLY! Not to be used on humans.**