





	<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.

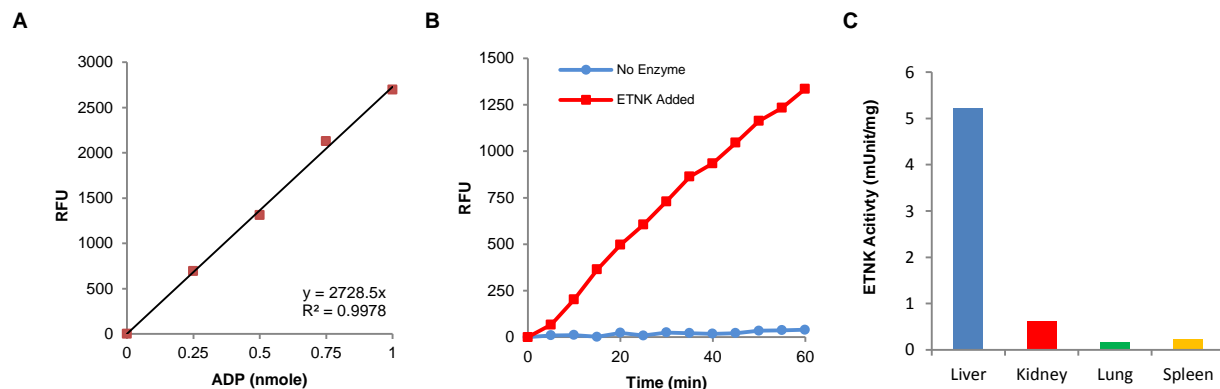
**4. Measurement:** Measure fluorescence at Ex/Em 535/587 nm in kinetic mode at 37°C for 60 min.

**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)	Choline Kinase B, Human Recombinant (P1220)
Phospholipid Assay Kit (Colorimetric/Fluorometric) (K351)	Phospholipase A2 Activity Assay Kit (Fluorometric) (K400)
Phospholipase D (PLD) Activity Colorimetric Assay Kit (K725)	Malate Colorimetric Assay Kit (K637)
Pyruvate Colorimetric/Fluorometric Assay Kit (K609)	PicoProbe™ Acetyl-CoA Fluorometric Assay Kit (K317)
Citrate Colorimetric/Fluorometric Assay Kit (K655)	Oxaloacetate Colorimetric/Fluorometric Assay kit (K659)
Citrate Synthase Activity Colorimetric Assay Kit (K318)	Isocitrate Colorimetric Assay Kit (K656)
Succinate (Succinic Acid) Colorimetric Assay Kit (K649)	

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