Enolase Activity Assay (Colorimetric and Fluorometric)

(Catalog: TBS2092, 100 Assays, Store at -20ºC)

DESCRIPTION

Enolase is a key glycolytic enzyme in the cytoplasm of cells. It catalyzes the dehydration of 2-phosphoglycerate (2-PG) to phosphoenolpyruvate (PEP), in the catabolic glycolytic pathway. Besides its glycolytic function, enolase plays a variety of roles in pathophysiological settings including oncogenesis, tumor progression, ischemia, and bacterial infection. In addition, neuron-specific enolase is released into the cerebrospinal fluid and the systemic circulation upon traumatic brain injury and ischemic episodes. Thus, the measurement of the enolase activity of enolase is a good predictor for patients to outcome post cardiac arrest.

Tribioscience's Enolase Activity Assay is designed to be a flexible, accurate, simple, and time-saving method in which enolase catalyzes 2-PG into PEP, resulting in an intermediate formation through a coupled enzyme reaction. It is catalyzed and reacts with the probe, to generate a colorimetric (570 nm) or fluorometric (Ex/Em=530/590 nm) intensity proportional to the enolase activity which can be measured.

APPLICATIONS

Direct Assays: Enolase activity in cells, serum and other biological samples.

KIT CONTENTS

Name	Size (100 tests)
Assay buffer	25 mL
Enolase Enzyme Mix	420 µL
Enolase Substrate	300 µL
Assay Cofactor	120 µL
Assay Developer	110 µL
Enolase Probe	60 µL
Enolase Positive Control	8 µL
Hydrogen Peroxide standard (0.88M)	10 µL

STORAGE AND HANDLING

Store kit at -20°C. Shelf life of 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 x 10^6) can be lyses 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 assays, the sample should be quickly frozen using liquid N_2 or dry ice if it is to be assayed later.

2. Colorimetric Standard Curve Preparations:

2.1 Dilute 2.0µL of 0.88M H_2O_2 into 218 µL of 1x Assay buffer to generate 8 mM H_2O_2 stock solution (*Note: 8mM H_2O_2 stock solution prepared in this step will be less stable and should be used within a few hours of preparation, although the H_2O_2 stock solution (0.88M) has been stabilized to slow its degradation).*

2.2 Label 1.5mL tubes 1-7 for a standard curve preparation displayed as Table 1.

Add 390µL of 1x Assay Buffer to Std 1 and 200µL to Std 2-7.

2.3 Add 10 µL of 8mM H_2O_2 Stock solution to Std1 then transfer 200µL of Std1 to Std2. Carry out a 2x serial dilution for Std 3-6. Leave Std 7 as the 0 standard (the assay buffer alone). The standards concentrations are 10, 5, 2.5, 1.25, 0.625, 0.313, 0.156, and 0 nmole/well for Std 1-7.

Table 1: Standard Preparation

Tubes	H_2O_2 (µL)	Buffer	H_2O_2 nmole/well
1	10 µL 8mM stock	390 µL	10
2	200 µL of Tube#1	200 µL	5
3	200 µL of Tube#2	200 µL	2.5
4	200 µL of Tube#3	200 µL	1.25
5	200 µL of Tube#4	200 µL	0.625
6	200 µL of Tube#5	200 µL	0.313
7	200 µL of Tube#6	200 µL	0.156
8	0	200 µL	0

3. Fluorometric Standard Preparation:

The H_2O_2 standard preparation is like the colorimetric assay, but this method is more sensitive than the colorimetric approach. The H_2O_2 concentration is adjusted as Table 2:

Table2: Fluorometric Assay Standard Preparation

Tubes	H_2O_2 (µL)	Buffer	H_2O_2 pmole/well
1	2 µL 8mM stock	398 µL	2000
2	200 µL of Tube#1	200 µL	1000
3	200 µL of Tube#2	200 µL	500
4	200 µL of Tube#3	200 µL	250
5	200 µL of Tube#4	200 µL	125
6	200 µL of Tube#5	200 µL	62.5
7	0	200 µL	0

Add 50 µL/well standards or samples to 96-well plate (Note: use black plate for fluorometric assay).

4. Enolase Positive Control:

Dilute Enolase Positive Control 50 times by adding 6 µL of the Positive Control to 294 µL Assay Buffer. Add 50 µL of the diluted positive control to indicated well.

5. Enolase Reaction Mix:

Prepare 50 µL of reaction mix for each test well as Table 3:

Table 3: Reaction Mix Preparation

Reaction Components	Volume
Enolase Assay Buffer	41 µL
Enzyme Mix	4 µL
Enolase Probe	0.5 µL
Enolase Substrate	2.5 µL
Assay Cofactor	1 µL
Assay Developer	1 µL
Final Volume (µL)	**50 µL**

Add 50 µL of the Enolase Reaction Mix to each well containing the enolase Standards, Positive Control, and test samples. Tap

plate lightly to mix. Protect the plate from light during incubation.

6. Measurement:
Measure the absorbance value at 570 nm (OD 570 nm) or fluorescence at Ex/Em = 530/590 nm in kinetic model for 5-15 min at 37°C (Note: the incubation time is dependent on the enolase activity in the samples). Choose two time points (T1 and T2) in the linear range to calculate the enolase activity of the samples.

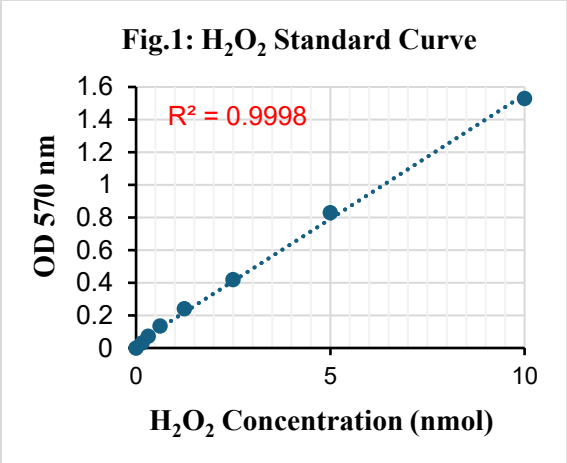
7. Calculation:
Correct background by subtracting the value of the 0 standard (blank) from all readings. Plot the standard curve. Apply the corrected sample value to the standard curve to get B nmole of H_2O_2 generated by enolase during the reaction time ΔT=T-T2.

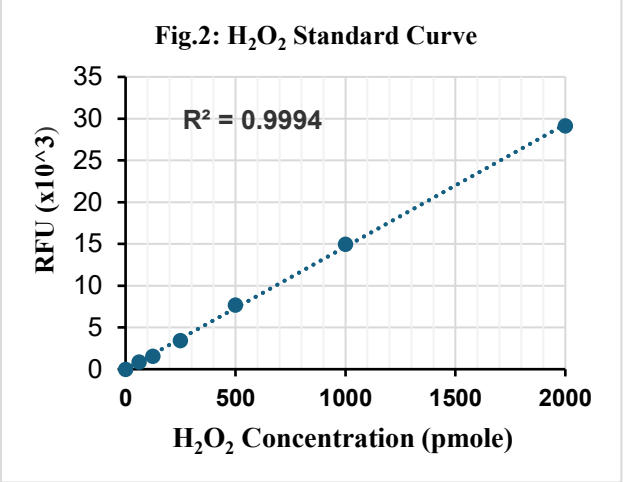
Sample Enolase Activity = N* B/(ΔT*V) = nmole/ul or pmol/min/uL= mU/ul or uU/ul = U/mL or mU/ml

Where: B=H_2O_2 amount from Standard Curve (nmole or pmole).
V= Sample volume in the reaction well (uL)
N= Dilution Factor.

Unit Definition: One milliunit (mU) is the amount of enzyme that will generate 1.0 nmole of H_2O_2 per minute at pH7.5 at 25 °C.

8. Typical Standard Curve for Colorimetric and Fluorometric Assays:
The typical standard curves are shown in Fig. 1 and Fig.2. The Typical standard curves are only used as a reference for assay. It cannot be used for real standard curve for assay.





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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)
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Pyruvate Colorimetric Assay (TBS2023C)
Glycerol Colorimetric Assay (TBS2204C)
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NNMT Activity Fluorometric Assay (TBS2098)
G6PDH Activity Colorimetric Assay (TBS2102
Cytochrome c Reductase Activity Assay (TBS2116)
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