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Triose Phosphate Isomerase Activity Colorimetric Assay Kit

6/14

(Catalog # K670-100; 100 assays; Store at -20°C)

I. Introduction:

Triose Phosphate Isomerase (TPI or TIM: EC 5.3.1.1) is an important enzyme for glycolysis. It reversibly interconverts dihydroxyacetone phosphate and glyceraldehyde-3-phosphate, thus maintaining the equilibrium of these two triose phosphates. TPI connects glycolysis to pentose phosphate pathway and lipid metabolism. It is a stable homodimer found in almost all organisms. In humans, TPI deficiency is a rare multisystem disorder and leads to progressive neurological dysfunction, characterized by hemolytic anemia, cardiomyopathy and progressive neuromuscular impairment. BioVision's Triose Phosphate Isomerase Activity Assay kit provides a quick and easy way for monitoring Triose Phosphate Isomerase activity in a variety of samples. In this kit, Triose Phosphate Isomerase converts dihydroxyacetone phosphate into glyceradehyde-3-phosphate, which reacts with the Enzyme Mix & Developer to form a colored product with strong absorbance at 450 nm. The assay is simple, sensitive, & high-throughput and can detect Triose Phosphate Isomerase activity as low as 40 mU/ml.

Dihydroxyacetone Phosphate $\xrightarrow{\text{Triose Phosphate Isomerase}}$ Glyceraldehyde -3-Phosphate $\xrightarrow{\text{Enzyme Mix + Developer}}$ Color (OD 450 nm)

II. Application:

- Measurement of TPI activity in various tissues and cells
- Analysis of glycolysis & pentose phosphate pathway and lipid metabolism

III. Sample Type:

- Animal tissues: muscle, liver, heart, kidney, etc.
- Cell culture: Adherent or suspension Cells
- Human serum or plasma

IV. Kit Contents:

Components	K670-100	Cap Code	Part Number
TPI Assay Buffer	25 ml	WM	K670-100-1
TPI Substrate (Lyophilized)	1 vial	Blue	K670-100-2
TPI Enzyme Mix (Lyophilized)	1 vial	Green	K670-100-3
TPI Developer (Lyophilized)	1 vial	Red	K670-100-4
NADH Standard (Lyophilized)	1 vial	Yellow	K670-100-5
TPI Positive Control (Lyophilized)	1 vial	Orange	K670-100-6

V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer (ELISA reader)

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the experiment.

- **TPI Assay Buffer:** Bring to room temperature before use. Store at -20°C or 4°C.
- **TPI Substrate:** Reconstitute with 220 µl dH_2O. Pipette up and down to dissolve completely. Store at -20°C. Use within two months.
- **TPI Enzyme Mix:** Reconstitute with 220 µl TPI Assay Buffer. Aliquot and store at -70°C. Keep on ice while in use. Use within two months.
- **TPI Developer:** Reconstitute with 220 µl dH_2O. Pipette up and down to dissolve completely. Store at -20°C. Use within two months.
- **NADH Standard:** Reconstitute with 400 µl dH_2O to generate 1.25 mM (1.25 nmol/µl) NADH Standard solution. Aliquot and store at -20°C. Keep on ice while in use. Use within two months.
- **TPI Positive Control:** Reconstitute with 200 µl dH_2O and mix thoroughly. Aliquot and store at –70°C. Keep on ice while in use. Use within two months.

VII. TPI Activity Assay Protocol:

1. **Sample Preparation:** Serum or plasma samples can be measured directly. For cells or tissue lysate, homogenize tissue (5 mg) or cells (1×10^6) with 100 µl ice cold TPI Assay Buffer, and keep on ice for 10 min. Centrifuge at 10,000 X g for 5 min. and collect the supernatant. Add 2-50 µl supernatant per well & adjust the volume to 50 µl/well with TPI Assay Buffer. For positive control, take 2-20 µl of TPI Positive Control into desired well(s) and adjust the volume to 50 µl with TPI Assay Buffer.

   Notes:

   a. For unknown samples, we suggest doing pilot experiment & testing several doses to ensure the readings are within the Standard Curve range.

   b. For samples having background, prepare parallel sample well(s) as sample background control(s).

2. **NADH Standard Curve:** Add 0, 2, 4, 6, 8 and 10 µl of 1.25 mM NADH Standard into a series of wells in a 96-well plate to generate 0, 2.5, 5.0, 7.5, 10, and 12.5 nmol/well of NADH Standard. Adjust the volume to 50 µl/well with TPI Assay Buffer.

3. Reaction Mix: Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µl Mix containing:

	Reaction Mix	Background Control Mix*
TPI Assay Buffer	44 µl	46 µl
TPI Enzyme Mix	2 µl	2 µl
TPI Developer	2 µl	2 µl
TPI Substrate	2 µl	----

Add 50 µl of the Reaction Mix to each well containing Standards, samples and Positive Control.

*For samples having high background, add 50 µl of Background Control Mix to sample background control well(s).

4. Measurement: Measure absorbance immediately at 450 nm in kinetic mode for 20-40 min. at 37°C.

Note: Incubation time depends on the TPI activity in the samples. We recommend measuring OD in kinetic mode, and choosing two time points (T_1 & T_2) in the linear range to calculate the TPI activity of the samples. The NADH Standard Curve can be read in endpoint mode (i.e., at the end of incubation time).

5. Calculation: Subtract 0 Standard reading from all readings. Plot the NADH Standard Curve. If sample background control reading is significant, correct sample background by subtracting the value derived from the background control reading from sample reading. Calculate the TPI activity of samples: $\Delta OD = A_2 - A_1$. Apply the ΔOD to NADH Standard Curve to get B nmol of NADH generated by TPI during the reaction time ($\Delta T = T_2 - T_1$).

$$\textbf{Sample TPI Activity} = B/(\Delta T \times V) \times D = \text{nmol/min/µl} = \text{mU/µl} = \text{U/ml}$$

Where: **B** is the NADH amount from Standard Curve (nmol)
ΔT is the reaction time (min.)
V is the sample volume added into the reaction well (µl)
D is the dilution factor

TPI activity can also be expressed in U/mg of sample.

Unit Definition: One unit of Triose Phosphate Isomerase is the amount of enzyme that generates 1.0 µmol of NADH per min. at pH 7.4 at 37°C.

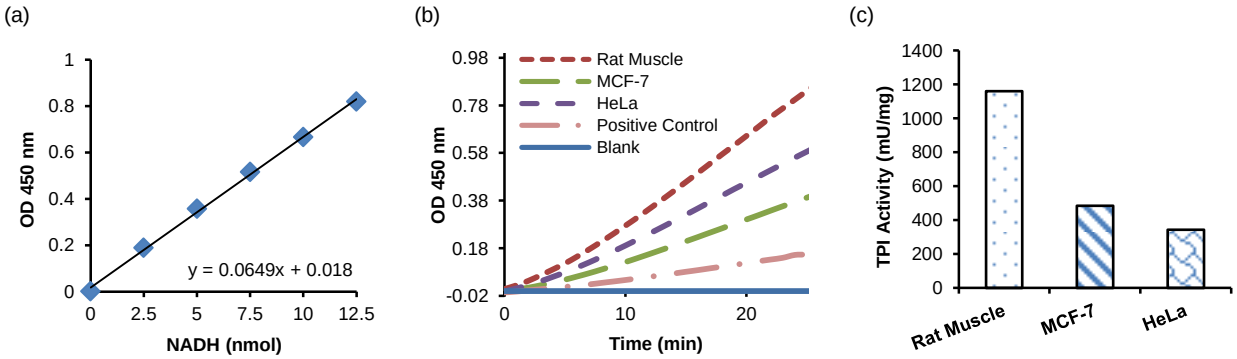

Figure: (a) NADH Standard Curve. (b) Kinetic measurement of Triose Phosphate Isomerase (TPI) activity in various samples. (c) Relative TPI activity was calculated in lysates prepared from rat muscle (0.5 µg), MCF-7 cells (0.8 µg), and HeLa cells (0.84 µg). Assays were performed following the kit protocol.

VIII. RELATED PRODUCTS:

Glucose Colorimetric Assay kit II (K686)
PicoProbe™ Glucose Fluorometric Assay Kit (K688)
Glucose-3-Phosphate Colorimetric Assay Kit (K641)
PEP Colorimetric/Fluorometric Assay Kit (K365)
Phosphoglucomutase Colorimetric Assay Kit (K774)
Pyruvate Colorimetric/Fluorometric Assay Kit (K609)
Glucose Uptake Colorimetric Assay Kit (K676)
Aldolase Activity Colorimetric Assay Kit (K665)
Glucose Colorimetric/Fluorometric Assay kit (K606)
2-Phosphoglycerate Colorimetric/Fluorometric Assay Kit (K778)
Hexokinase Colorimetric Assay Kit (K789)
Phosphofructokinase (PFK) Activity Colorimetric Assay Kit (K776)
Phosphoglucose Isomerase Colorimetric Assay Kit (K775)
Pyruvate Kinase Activity Colorimetric/Fluorometric Assay Kit (K709)
Glucose Uptake Fluorometric Assay Kit (K666)

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