3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Triose Phosphate Isomerase Activity Colorimetric Assay Kit

Catalog Number **MAK274**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Triose Phosphate Isomerase (EC 5.3.1.1, TPI, or TIM) is an important enzyme for glycolysis. It reversibly interconverts dihydroxyacetone phosphate and glyceraldehydes 3-phosphate, thus maintaining the equilibrium of these two triose phosphates. TPI connects glycolysis to the 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.

This Triose Phosphate Isomerase Activity Assay kit provides a quick and easy way for determining 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 and Developer to form a colored product with strong absorbance at 450 nm. The assay is simple, sensitive, and high-throughput, and can detect Triose Phosphate Isomerase activity as low as 40 mU/mL.

Components

The kit is sufficient for 100 assays in 96 well plates.

TPI Assay Buffer Catalog Number MAK274A	25 mL
TPI Substrate Catalog Number MAK274B	1 vial
TPI Enzyme Mix Catalog Number MAK274C	1 vial
TPI Developer Catalog Number MAK274D	1 vial
NADH Standard Catalog Number MAK274E	1 vial
TPI Positive Control Catalog Number MAK274F	1 vial

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – clear plates are recommended for this assay.
- Spectrophotometric multiwell plate reader

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge all small vials prior to opening. Use ultrapure water for the preparation of reagents.

TPI Assay Buffer – Bring to room temperature before use. Store at –20 °C or 4 °C.

TPI Substrate – Reconstitute with 220 µL of water. Pipette up and down to dissolve completely. Store at –20 °C. Use within two months.

TPI Enzyme Mix – Reconstitute with 220 µL of 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 of water. Pipette up and down to dissolve completely. Store at –20 °C. Use within two months.

NADH Standard – Reconstitute with 400 µL of water to generate 1.25 mM (1.25 nmole/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 of water and mix thoroughly. Aliquot and store at –70 °C. Keep on ice while in use. Use within two months.

Storage/Stability
The kit is shipped on wet ice and storage at –20 °C, protected from light, is recommended. Briefly centrifuge all small vials prior to opening.

Procedure
All samples and standards should be run in duplicate.

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 of ice cold TPI Assay Buffer, and keep on ice for 10 minutes. Centrifuge at 10,000 × g for 5 minutes and collect the supernatant. Add 2–50 µL of supernatant per well and 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:
For unknown samples, doing a pilot experiment and testing several doses to ensure the readings are within the Standard Curve range is suggested.

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

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 nmole/well of NADH Standard. Adjust the volume to 50 µL/well with TPI Assay Buffer.

Reaction Mixes
Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µL of the appropriate Mix, see Table 1.

Table 1
Preparation of Mixes

Reagent	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).

Measurement
Measure absorbance immediately at 450 nm in kinetic mode for 20–40 minutes at 37 °C.

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

Results
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: $\Delta OD = A_2 - A_1$. Compare the ΔOD to the NADH Standard Curve to obtain the amount of NADH (B, in nmole) generated by TPI during the reaction time ($\Delta T = T_2 - T_1$).

$$\text{TPI Activity} = B/(\Delta T \times V) \times D$$

B = the NADH amount from Standard Curve (nmole)
ΔT = the reaction time (minutes)
V = the sample volume added into the reaction well (µL)
D = the dilution factor

TPI activity can also be expressed in U/mg of sample:
(nmole/min/µL = mU/µL = U/mL)

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

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay Not Working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	Clear plates are recommended for this assay.
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

SJ,MAM 04/16-1