Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

Transketolase Activity Assay Kit (Fluorometric)

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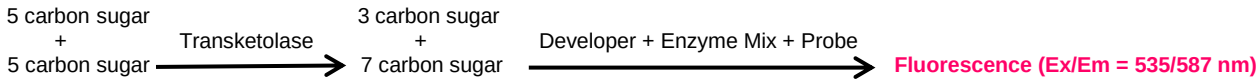
(Catalog # K2004-100; 100 assays; Store at - 20ºC)

I. Introduction:

Transketolase (EC 2.2.1.1) is an important enzyme of the non-oxidative branch of the pentose phosphate pathway, which metabolizes glucose to form pentose and NADPH. It is also involved in the photosynthetic Calvin cycle in plants and autotrophic bacteria. Transketolase (TKT) catalyzes two reactions in the pentose phosphate pathway, both of which are involved in the transfer of a 2-carbon glycoaldehyde fragment from a α-keto pentose sugar such as xylulose-5-phosphate to another aldose sugar such as ribose-5-phosphate or erythrose-4-phosphate. It is present in the cytosol of most tissues and its activity depends on the binding of thiamin pyrophosphate, a derivative of thiamin (Vitamin B1). Therefore TKT activity is decreased in thiamine deficiency and may be used in the diagnosis of Wernicke-Korsakoff syndrome. BioVision's Transketolase Activity Assay Kit is a simple, plate-based fluorometric assay for measuring TKT activity in biological samples. In this assay, TKT transfers a two-carbon group from a donor keto sugar to an acceptor aldose sugar. The product formed converts a non-fluorescent probe to a fluorescent product via an enzymatic reaction in the presence of a developer and an enzyme mix. The assay can detect as low as 5 µU of TKT activity in biological samples.



II. Applications:

Measure Transketolase activity

III. Sample Type:

- Tissue lysate (e.g. Liver tissue)
- Cell lysate
- Recombinant enzyme
- Purified protein

IV. Kit Contents:

Components	K2004-100	Cap Code	Part Number
TKT Assay Buffer	35 ml	NM	K2004-100-1
TKT Reconstitution Buffer	200 µl	Amber	K2004-100-2
TKT Substrate Mix	1 vial	White	K2004-100-3
TKT Developer	1 vial	Green	K2004-100-4
TKT Enzyme Mix	1 vial	Red	K2004-100-5
TKT Probe	400 µl	Blue	K2004-100-6
Glyceraldehyde 3-phosphate Standard	1 vial	Yellow	K2004-100-7
TKT Positive Control	1 vial	Purple	K2004-100-8

V. User Supplied Reagents and Equipment:

- 96-well white plate with flat bottom
- Multi-well spectrophotometer
- Distilled water
- 10 kDa Spin Columns (BV# 1997)

VI. Storage Conditions and Reagent Preparation:

Upon arrival, store the kit at -20°C, protected from light. Briefly centrifuge small vials before opening. Read the entire protocol before performing the assay. Components are stable for at least three months.

- **TKT Assay Buffer:** Warm to room temperature (RT) before use.
- **TKT Reconstitution Buffer:** Keep on ice when in use.
- **TKT Substrate Mix, TKT Developer and Enzyme Mix:** Reconstitute each in 220 µl TKT assay buffer. Aliquot and store at -20ºC in the dark. Thaw on ice before use.
- **Glyceraldehyde 3-Phosphate Standard:** Reconstitute in 1.5 ml water to obtain a 20 mM Glyceraldehyde 3-Phosphate (G3P) Standard solution. Aliquot and store at -20°C. Thaw at RT before use.
- **TKT Probe:** Thaw at RT.
- **TKT Positive Control:** Reconstitute the vials in 44 µl TKT Reconstitution buffer. Store at -20ºC and always keep on ice when in use. Mix by pipetting very gently. **Lyophilized TKT is stable for 12 months and for at least 2 months after reconstitution.**

VII. Transketolase Activity Assay Protocol:

1. Sample preparation: Homogenize cells (4 x 10^5 cells) or tissue (10 mg) with 100 µl TKT Assay buffer to perform lysis. Keep on ice for 10 min followed by centrifugation at 10,000 x *g* and 4ºC for 15 min. Collect the lysate supernatant and estimate the protein concentration using any preferred method. We recommend using BCA protein assay kit (BV# K813-2500). Protein concentration should range between 0.05-0.2 µg/µl for tissue lysates and between 1-4 µg/µl for cell lysates. Dilute the lysate if needed using TKT Assay Buffer. Prepare two wells for each sample to be tested labeled as Sample Background Control (**SBC**), and Sample (**S**). Add 2-4 µl Sample (up to 0.6 µg protein for tissue lysates and up to 8 µg protein for cell lysates) into each of these wells. For Positive Control, add 4 µl of the TKT Positive Control into the desired well(s). Adjust the volume to 50 µl/well with TKT Assay Buffer. For Substrate Control wells, add 50 µl of TKT Assay Buffer.

Notes:

a) For Sample(s) with high background such as liver tissue lysate, dilute the lysate with TKT assay buffer 5-10 times and filter through 10 kDa Spin Columns (BV# 1997). Small molecules will be removed in the ultrafiltrate, which is used for the TKT activity assay.

b) We recommend using the Samples for activity analysis immediately. Otherwise, store the Sample(s) at -80ºC for 3-4 days.

c) For Unknown Samples, we suggest testing several dilutions to ensure that the readings are within the Standard Curve range.

2. Glyceraldehyde 3-Phosphate (G3P) Standard Curve: Dilute the reconstituted 20 mM G3P Standard solution at 1:800 (20 times followed by 40 times dilution) in TKT assay buffer to obtain 25 µM G3P Standard solution. Add 0, 2, 4, 8, 12 and 16 µl of the 25 µM G3P Standard solution into a series of wells in a 96-well white plate to obtain 0, 50, 100, 200, 300 and 400 pmol/well G3P Standard respectively. Adjust the volume of each well to 50 µl with TKT Assay Buffer.

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

	Reaction Mix	Background Mix
TKT Assay Buffer	42 µl	44 µl
TKT Substrate Mix	2 µl	-
TKT Developer	2 µl	2 µl
TKT Enzyme Mix	2 µl	2 µl
TKT Probe	2 µl	2 µl

Mix well and add 50 µl Reaction Mix to wells of a 96-well white plate containing Substrate Control, Sample, and Positive Control. Add 50 µl Background Mix to wells containing G3P Standard and SBC. Mix well.

Notes:

a) Have the microplate reader ready at Ex/Em 535/587 nm in kinetic mode at 37ºC set to record fluorescence every 30 sec.

b) Prepare Reaction Mix immediately before adding to the wells.

4. Measurement: Immediately start recording fluorescence at 30 sec intervals for 30-45 min at 37ºC. Standard Curve may be read in either kinetic or end point mode (after 40 min).

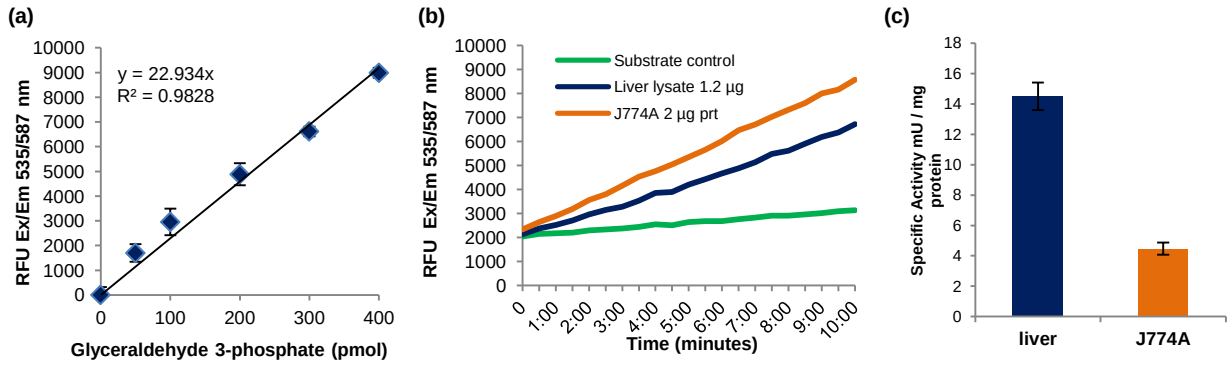
5. Calculation: Subtract the 0 Standard readings from all Standard readings and SBC readings from Sample readings respectively. Plot the G3P Standard Curve. Choose any two time points within the linear portion of the curve ($\mathbf{t_1}$ & $\mathbf{t_2}$) for each Sample type. Subtract the SBC readings from the corresponding Sample readings for the chosen $\mathbf{t_1}$ & $\mathbf{t_2}$ time points. If the Substrate Control reading is higher than the SBC reading, subtract the Substrate Control readings from the Sample readings instead. Apply the corrected Sample readings to the G3P Standard Curve to get **ΔM** pmol of G3P formed during the reaction time ($\Delta \mathbf{t} = \mathbf{t_2} - \mathbf{t_1}$).

Calculate the TKT activity of the Samples using the following equation:

$$\textbf{Sample TKT Specific Activity} = \Delta M \times D / (\Delta t \times P) \text{ (pmol / (min x µg))} = \text{µUnits/µg or mUnits/mg}$$

Where: **ΔM =** G3P conc from the Standard Curve (pmol)
$\Delta \mathbf{t} = t_2 - t_1$ (min)
D = Sample dilution factor
P = Sample used (in µg)

Unit Definition: One unit of Transketolase is the amount of enzyme that produces1 µmol of G3P per minute at pH 7.5 at 37ºC.



Figures: (**a**) Gyceraldehyde 3-phosphate Standard Curve (**b**) Enzyme kinetics using rat liver lysate (1.2 µg protein/well) and J774A cell lysate (2 µg protein/well) **(c)** TKT specific activity in rat liver lysate and J774A cells. Experiments were conducted according to kit protocol.

VIII. Related Products:

PicoProbe™ Glucose-6-Phosphate Fluorometric Assay Kit (K687)
6-Phosphogluconate Dehydrogenase Activity Colorimetric Assay Kit (K540)
6-Phosphogluconic Acid (6-PGA) Assay Kit (Colorimetric) (K217)
Triose Phosphate Isomerase (TPI) Activity Colorimetric Assay Kit (K670)

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