Technical Bulletin

Transketolase Activity Assay Kit (Fluorometric)

Catalog Number MAK420

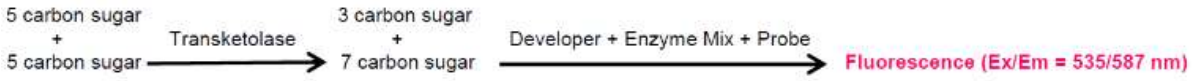
Product Description

Transketolase (TKT) is an important enzyme of the non-oxidative branch of the pentose phosphate pathway, which metabolizes glucose to form pentose and NADPH. TKT is also involved in the photosynthetic Calvin cycle in plants and autotrophic bacteria. Transketolase catalyzes two reactions in the pentose phosphate pathway, both of which are involved in the transfer of a 2-carbon glycoaldehyde fragment from an α-keto pentose sugar, such as xylulose-5-phosphate, to another aldose sugar, such as ribose-5-phosphate or erythrose-4-phosphate. TKT is present in the cytosol of most tissues and its activity depends on the binding of thiamin pyrophosphate, a derivative of thiamin (Vitamin B_1).

Therefore, TKT activity is decreased in thiamine deficiency and may be used in the diagnosis of Wernicke-Korsakoff syndrome.

The Transketolase Activity Assay Kit is a simple 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 (λ_{Ex} = 535 nm/λ_{Em} = 587 nm) 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.

The kit is suitable for the measurement of transketolase activity in tissue lysate (e.g., liver), cell lysate, recombinant enzymes, and purified proteins.



Components

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

- TKT Assay Buffer — 35 mL
  Catalog Number MAK420A
- TKT Reconstitution Buffer — 200 μL
  Catalog Number MAK420B
- TKT Substrate Mix — 1 vial
  Catalog Number MAK420C
- TKT Developer — 1 vial
  Catalog Number MAK420D
- TKT Enzyme Mix — 1 vial
  Catalog Number MAK420E
- TKT Probe — 400 μL
  Catalog Number MAK420F
- Glyceraldehyde 3-phosphate Standard — 1 vial
  Catalog Number MAK420G
- TKT Positive Control — 1 vial
  Catalog Number MAK420H

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Fluorescence multiwell plate reader
- White flat-bottom 96-well plates. Cell culture or tissue culture treated plates are **not** recommended.
- Dounce tissue grinder set (Catalog Number D9063 or equivalent)
- Refrigerated microcentrifuge capable of RCF ≥10,000 × *g*
- Corning® Spin-X® UF concentrators (Catalog Number CLS431478)
- Bicinchoninic Acid Kit for Protein Determination (Catalog Number BCA1 or equivalent)

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C, protected from light.

Preparation Instructions

Briefly centrifuge small vials prior to opening.

TKT Assay Buffer: Ready to use. Warm to room temperature prior to use.

TKT Reconstitution Buffer: Ready to use. Keep on ice when in use.

TKT Substrate Mix, TKT Developer and Enzyme Mix: Reconstitute each vial in 220 µL TKT Assay Buffer. Aliquot and store at -20 °C, protected from light. Thaw on ice prior to use.

Glyceraldehyde 3-Phosphate Standard: Reconstitute vial in 1.5 mL water to obtain a 20 mM Glyceraldehyde 3-Phosphate (G3P) Standard solution. Aliquot and store at -20 °C. Thaw at room temperature prior to use.

TKT Probe: Ready to use. Thaw at room temperature.

TKT Positive Control: Reconstitute the vial in 44 µL of TKT Reconstitution buffer. Store at -20 °C and always keep on ice when in use. Mix by pipetting very gently. Lyophilized TKT is stable for twelve months and for at least two months after reconstitution.

Procedure

All samples and standards should be run in duplicate.

Sample Preparation

1. Homogenize cells (4×10^5 cells) or tissue (10 mg) with 100 µL of TKT Assay Buffer to perform lysis.
2. Keep on ice for 10 minutes followed by centrifugation at 10,000 *x g,* 4 °C for 15 minutes.
3. Collect the lysate supernatant and estimate the protein concentration using BCA protein determination.
4. For samples with high background such as liver tissue lysate, dilute the lysate with TKT assay buffer 5-10 times and filter through a 10 kDa Spin Column such as Corning Spin-X UF concentrator. Small molecules will be removed in the ultrafiltrate, which is used for the TKT activity assay.
5. 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.
6. Use the prepared samples for activity analysis immediately. Otherwise, store the samples at -80 °C for 3-4 days.

7. Prepare two wells for each sample to be tested labeled as Sample Background Control (SBC), and Sample (S).
8. 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 unknown samples, test several dilutions to ensure that the readings are within the Standard Curve range.
9. Adjust the total volume to 50 µL/well with TKT Assay Buffer.

Positive Control (PC)

Add 4 µL of the TKT Positive Control into the desired well(s). Adjust the total volume to 50 µL/well with TKT Assay Buffer.

Substrate Control (SC)

Add 50 µL of TKT Assay Buffer into the desired well(s). Adjust the total volume to 50 µL/well with TKT Assay Buffer.

Standard Curve Preparation

1. Prepare a 1 mM G3P Standard solution by diluting the reconstituted 20 mM G3P Standard 1:20 with TKT Assay Buffer.
2. Prepare a 25 µM G3P Standard solution by diluting the 1 mM G3P Standard solution from Step 1 1:40 with TKT Assay Buffer.
3. Prepare Standards according to Table 1. Mix well.

Table 1.
Preparation of G3P Standards

Well	25 µM G3P Standard	TKT Assay Buffer	G3P (pmol/well)
1	0 µL	50 µL	0
2	2 µL	48 µL	50
3	4 µL	46 µL	100
4	8 µL	42 µL	200
5	12 µL	38 µL	300
6	16 µL	34 µL	400

Instrument Setup

Preset the microplate reader at λ_{Ex} = 535 nm/ λ_{Em} = 587 nm in kinetic mode at 37 °C set to record fluorescence every 30 seconds.

Reaction Mixes

1. Prepare Reaction Mixes immediately before adding to the wells. Mix enough reagents for the number of assays to be performed.
   a. For each well containing Substrate Control (SC), Sample (S), and Positive Control (PC), prepare 50 µL of Reaction Mix according to Table 2, mix well.
   b. For each G3P Standard and Sample Background Control (SBC) well, prepare 50 µL of Background Reaction Mix according to Table 2. Mix well.

Table 2.
Preparation of Reaction Mixes

Reagent	Reaction Mix	Background Reaction 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

2. Add 50 µL of Reaction Mix to wells of a 96-well white plate containing Substrate Control, Sample, and Positive Control. Mix well.

3. Add 50 µL of Background Mix to wells containing G3P Standard and SBC. Mix well.

Measurement

Immediately start recording fluorescence (RFU) (λ_{Ex} = 535 nm/λ_{Em} = 587 nm) at 30 second intervals for 30-45 minutes at 37 °C. Standard Curve may be read in either kinetic or end point mode (after 40 minutes).

Results

1. Subtract the 0 Standard RFU readings from all Standard readings.
2. Plot the G3P Standard Curve.
3. Choose any two time points within the linear portion of the curve (T_1 and T_2) for each Sample (S).
4. Subtract the Sample Background Control (SBC) RFU readings from the corresponding Sample RFU readings for the chosen T_1 and T_2 time points. If the Substrate Control (SC) RFU reading is higher than the SBC reading, subtract the Substrate Control readings from the Sample readings instead.
5. Apply the corrected Sample RFU readings to the G3P Standard Curve to get M pmol of G3P formed during the reaction time ($\Delta T = T_2 - T_1$).
6. Calculate the TKT activity of the Samples using the following equation:

TKT Specific Activity (pmol / (min × μg) or μUnits/μg or mUnits/mg) =

$$(M \times D) / (\Delta T \times P)$$

where

M = Amount of G3P from the Standard Curve (pmol)

D = Sample dilution factor (for undiluted samples, D = 1)

ΔT = $T_2 - T_1$ (minutes)

P = Sample used (in μg protein)

Unit Definition: One unit of Transketolase is the amount of enzyme that produces 1 μmol of G3P per minute at pH 7.5 at 37 °C.

Figure 1.
Typical Glyceraldehyde 3-phosphate (G3P) Standard Curve

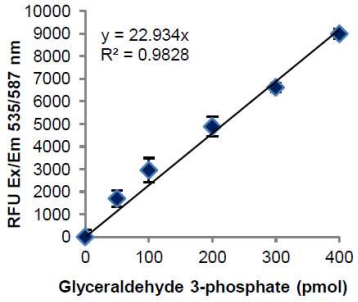


Figure 2.
Enzyme kinetics using rat liver lysate (1.2 μg protein/well) and J774A cell lysate (2 μg protein/well)

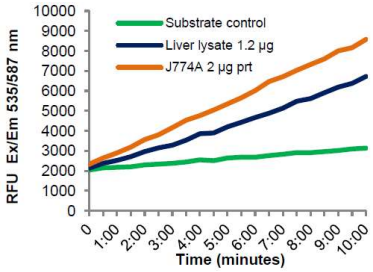
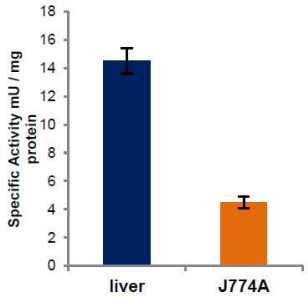


Figure 3.
TKT specific activity in rat liver lysate and J774A cells. Assays were conducted according to kit protocol.



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